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THE RELATIONSHIP OF GASTRIC ACTIVITY AND ADRENOCORTICAL FUNCTION: A CORRELATIVE STUDY*

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INTRODUCTION

STUDIES on human subjects reported previously from this laboratory have indicated that the administration of corticotropin or adrenal glucocorticoids may induce a marked increase in basal and nocturnal gastric acid and pepsin secretion, with a concomitant increase in urinary uropepsin (1–5).

The administration of adrenal glucocorticoids has resulted in the development of epigastric pain or peptic ulcer in patients without previous gastrointestinal disease, and has led to reactivation of quiescent peptic ulcer in others (5–7). In patients with Addison's disease, in whom peptic ulceration is exceedingly rare, the administration of cortisone in the small replacement dosage of 25 to 50 mg. daily has been followed by documented gastric and duodenal ulcer formation (8, 9).

Further indirect evidence favoring the hypothesis that gastric and adrenal activity are closely related is derived from study of the uropepsin

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and urinary steroid excretion in patients with hypofunction or hyperfunction of the adrenal and pituitary glands. A markedly diminished uropepsin excretion in patients with adrenal hypofunction (panhypopituitarism and Addison's disease) and a definite increase in uropepsin excretion in adrenal hyperfunction (Cushing's disease) have been noted, with parallel alterations in the urinary corticoid and ketosteroid excretions (5).

Administration of corticotropin to Heidenhain-pouch dogs induced a rise in gastric acid, pepsin and chloride, and a fall in sodium and potassium, confirming previous studies in man (10). That corticotropin may induce an increase in gastric acid and pepsin in the experimental animal has been noted by others (11–13).

It is the purpose of this study to investigate the significance of the adrenal-gastric relationship in normal man before and after corticotropin stimulation, by statistical application of coefficients of correlation. Uropepsin excretion is utilized as an index of gastric secretory function, and urinary 17-hydroxycorticoid and 17-ketosteroid excretions are applied as indices of adrenal activity.

MATERIALS AND METHODS

A. *Biochemical methods*

1. *Urinary uropepsin.* Urinary uropepsin was determined by a modification of the method of Bucher, as reported previously (14). Uropepsin excretion has been shown to be an accurate index of gastric-juice pepsin, representing a fraction of the gastric pepsin

TABLE 1. RELATIONSHIP OF UROPEPSIN TO GASTRIC PEPSIN DURING CHRONIC STIMULATION WITH CORTICOTROPIN (ACTH)

Patient	Control period			Maximum response to ACTH stimulation		
	Gastric pepsin (units/hr.)	Uropepsin (units/hr.)	Uropepsin / pepsin	Gastric pepsin (units/hr.)	Uropepsin (units/hr.)	Uropepsin / Pepsin
1	18,500	146	0.8%	56,000	468	0.8%
2	36,800	113	0.3%	63,000	218	0.3%
3	48,000	203	0.5%	120,000	428	0.4%
4	15,600	163	1.0%	69,300	483	0.9%
5	39,000	100	0.3%	99,700	242	0.2%
6	13,900	133	1.0%	41,500	329	0.8%

which remains relatively constant for each individual (5, 15). Corticotropin does not alter the uropepsin-pepsin ratio, indicating that the adrenal steroids do not change the pepsinogen partition at the gastric level or alter the renal threshold for pepsinogen (Table 1).

2. *Urinary 17-hydroxycorticoids.* Urinary 17-hydroxycorticoids were determined by a modification of the method of Reddy, Jenkins and Thorn (16).

3. *Urinary 17-ketosteroids.* Urinary 17-ketosteroids were determined in accordance with the method of Drekter (17); colorimetry was performed as in the procedure of Holtorff and Koch (18).

B. *Statistical methods*

1. *Coefficients of correlation*

a. Total *r*. The degree of association between two measurements (or variables) taken on the same subjects was measured by the "product-moment correlation coefficient" symbolized by "*r*" (19). This coefficient may assume any value between -1 and $+1$. A value of $+1$ indicates a perfect association between the two characteristics in the observed set of measurements, a value of 0 indicates a complete absence of any association in them, and a value of -1 a perfect inverse relationship.

b. Partial *r*. A total correlation coefficient measured the association between two variables as they occurred in the data. In order to evaluate the relationship among two of the variables independent of the third one, partial correlations were also calculated (20).

2. *Z test of Fisher.* Because of the variability of samples it is possible to obtain relatively large values of *r* in data when the "true" correlation in the population, P, is zero. A *t* test may be made on any correlation coefficients to test the null hypothesis $P=0$ by taking

$$t = r\sqrt{\frac{N-2}{1-r^2}}, \text{ d.f.} = N-2.$$

However, any other inferences regarding the value of P are limited by the fact that *r* values obtained in small samples from a population in which P is moderately large are asymmetrically distributed. The *r* values were therefore transformed into corresponding "*z*" values ($z=[\frac{1}{2}\log_e(1+r)-\log_e(1-r)]$) by Fisher's *z* transformation (21, 22), and tests of homogeneity or significance were performed on these *z* values.

C. *Plan of study*

Statistical correlations between urinary uropepsin and urinary 17-hydroxycorticoids and 17-ketosteroids were studied in normal subjects, before and following administration of corticotropin.

1. *Unstimulated subjects.* Twenty-four-hour urine collections were obtained from 11 normal subjects ranging in age from 20 to 45, for periods of eight to forty-four days. Each sample was analyzed for uropepsin, 17-hydroxycorticoids and 17-ketosteroids. Correlation coefficients were subsequently determined for the following three relationships: a) uropepsin versus 17-hydroxycorticoids, b) uropepsin versus 17-ketosteroids, and c) 17-hydroxycorticoids versus 17-ketosteroids.

2. *Exogenous corticotropin (ACTH) stimulation.* Two normal male subjects aged 22 were given intramuscular ACTH-gel in a dosage of 40 units twice daily for five consecutive days, preceded by a control period of five days. The period of stimulation was followed by a further control period of eight days. Urines were collected for consecutive twelve-hour periods throughout the study. Correlation coefficients relating uropepsin to 17-hydroxycorticoids, uropepsin to 17-ketosteroids, and 17-hydroxycorticoids to 17-ketosteroids were determined for the control periods and the period of corticotropin stimulation.

Three additional normal male subjects aged 40, 37 and 29 were likewise subjected to corticotropin stress. They were given an eight-hour intravenous infusion daily containing 25 units of ACTH in 1000 cc. of 5 per cent glucose and water, for five, six and seven days, respectively. This was preceded and followed by appropriate control periods.

In the ideal sense it would have been desirable to compare all of the subjects receiving corticotropin with themselves as unstimulated subjects, in accordance with the concept

of paired samples. Two of the corticotropin-treated subjects (Nos. 12 and 13 of Table 2) were part of the unstimulated group (Subjects 1 and 2). However, the large number of observations required to apply the coefficients of correlation properly necessitated the use of the two groups (unstimulated subjects and corticotropin-treated subjects), essentially composed of individuals unrelated to one another.

3. *Correlations in group data.* In 8 of the normal males (Subjects 4–11 of Table 2) correlation coefficients on the total group data were obtained. These correlations were calculated for all data obtained for the group as a whole, for each of the periods in question. This was considered valid, because there were equal numbers of observations for each of the 8 individuals and because the environment was controlled. These 8 subjects were isolated in a training camp for one month prior to a major crew race, and thus there was the uniformity of diet, emotional climate and physical activity that an athletic training camp provides. Correlation coefficients on the total data of this group for each of the three relationships previously mentioned were determined, both including and excluding the physical and emotional stress periods of a time trial and a final varsity race. The complete study of this crew, primarily in regard to the adrenocortical and psychologic response to stress, has been reported in detail previously (23).

RESULTS

A. *Normal unstimulated subjects*

The coefficients of correlation are summarized in Table 2.

In the 11 normal subjects without corticotropin stimulation the co-

TABLE 2. CORRELATION COEFFICIENTS RELATING GASTRIC AND ADRENAL FUNCTION

Normal subjects	No. of observations	Correlation coefficients		
		Uropepsin-17 OH	Uropepsin-17 KS	17 OH–17 KS
1	10	0.81*	0.80*	0.67†
2	8	0.48	0.64	0.79*
3	44	0.52*	0.57*	0.40*
4	12	0.54	−0.14	0.28
5	8	−0.15	0.41	0.81†
6	12	0.54	0.57	0.41
7	12	−0.02	−0.11	0.18
8	10	0.05	0.75†	0.40
9	8	0.28	0.46	0.56
10	12	−0.01	−0.12	0.29
11	12	0.22	0.72†	0.27
		Following corticotropin (ACTH)		
12	10	0.27	0.86*	−0.2
13	10	0.82*	0.74†	0.85†
14	5	—	0.68	—
15	7	—	0.89*	—
16	6	0.83†	—	—

* Significant at 1% level.
† Significant at 5% level.

efficients of correlation of the three relationships tested varied from −0.15 to +0.81. Subjects 1, 3, 8 and 11 demonstrated significant correlations ($P < .05$) between uropepsin and 17-ketosteroids. Significant correlations were present for uropepsin and 17-hydroxycorticoids in Subjects 1 and 3. The correlation between 17-ketosteroids and 17-hydroxycorticoids was significant ($P < .05$) in Subjects 1, 2, 3 and 5. Significant r values for any given correlation were not necessarily accompanied by significant correlation coefficients for the other relationships in that same individual.

TABLE 3. MEAN RESPONSE OF UROPEPSIN AND URINARY ADRENAL STEROID EXCRETION TO EXOGENOUS CORTICOTROPIN (ACTH)

Subject	Determination	Control	ACTH period	Control
12	UP	2889	4122	3094
	17 OH	6.8	17.5	6.8
	17 KS	27.4	38.5	24.8
13	UP	3200	4786	2071
	17 OH	7.1	22.5	7.6
	17 KS	21.2	38.6	25.3
14	UP	3494	10119	4494
	17 OH	—	—	—
	17 KS	9.1	24.6	8.4
15	UP	2803	7646	3078
	17 OH	—	—	—
	17 KS	7.3	23.5	7.0
16	UP	3996	7731	3850
	17 OH	1.0	38.3	3.6
	17 KS	—	—	—

UP = Uropepsin (units/24 hrs.)
17 OH = 17-Hydroxycorticoids (mg./24 hrs.)
17 KS = 17-Ketosteroids (mg./24 hrs.)

The relationships studied in these subjects had levels of significance more often than chance would permit, yet they demonstrated a less constant pattern than the correlation coefficients following administration of corticotropin. Although they did not conform to a truly random pattern, variation was present both between different individuals and within each individual for the three relationships in question.

B. *Normal subjects receiving corticotropin (ACTH)*

Following administration of corticotropin in the 2 normal subjects receiving intramuscular ACTH (Subjects 12 and 13, Tables 2 and 3), a

prompt and uniform response of uropepsin excretion, 17-hydroxycorticoid excretion, and 17-ketosteroid excretion occurred from the first day and throughout the period of stimulation (Table 3; Figs. 1 and 2).

In the 3 subjects receiving intravenous ACTH (Subjects 14, 15 and 16, Tables 2 and 3), uropepsin and 17-ketosteroid excretion had been determined in 2, and uropepsin and 17-hydroxycorticoid excretion in 1. The data for these subjects are tabulated in Table 3.

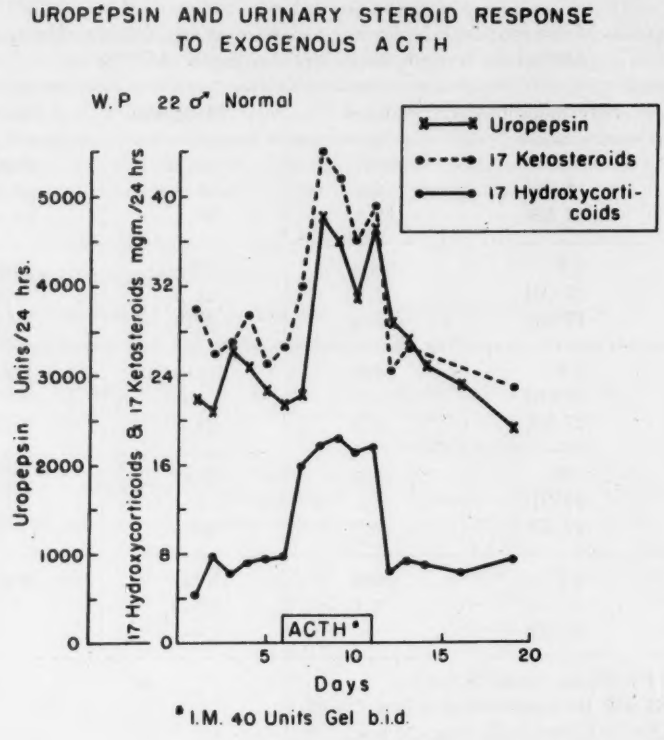

FIGURE 1

Correlation coefficients determined in the 5 normal subjects during the period of corticotropin administration demonstrated generally high values. This was especially noted in the correlation between uropepsin and 17-ketosteroids. In contrast, a more random pattern of significant correlations was suggested in the unstimulated normal group (Table 2).

As noted in statistical methods, since the spectrum of r values does not conform to a normal distribution curve, it was necessary to convert the simple correlation coefficient, r, to the z value, using a weighted z table to

compare the correlation coefficients in the control period with those in the corticotropin period (Table 4). The mean weighted z value for the unstimulated subjects was 0.52, compared to a mean weighted z value of 1.15 for the corticotropin period. The mean z values were significantly different ($P < 0.01$), demonstrating a higher z correlation for the period of

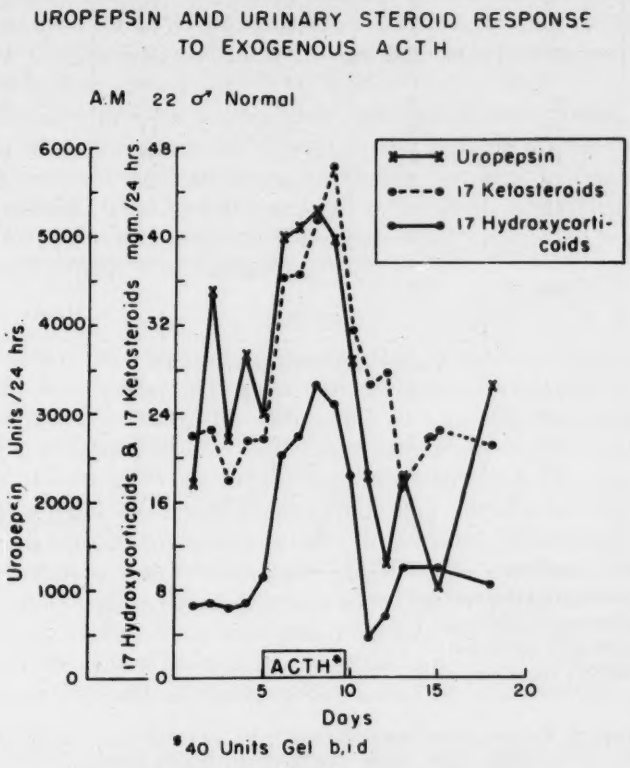

FIGURE 2

corticotropin stimulation. This conversion was carried out for the uropepsin-ketosteroid correlation alone. The large number of observations required by the statistical method precluded the possibility of documenting any significant relationship for the uropepsin-hydroxycorticoid or hydroxycorticoid-ketosteroid correlations for the period of corticotropin stimulation, although gross observation of the data suggests that the results for these two correlations might be similar to those noted for the uropepsin-ketosteroid correlation coefficients.

TABLE 4. COEFFICIENTS OF CORRELATION AND *z* VALUES FOR UROPEPSIN-17 KETOSTEROID CORRELATION BEFORE AND AFTER CORTICOTROPIN (ACTH)

Normal subjects	N [1]	DF [2]	*r* [3]	*z* [4]	Weighted *z* [2]×[4]
1	44	41	.57	.65	26.65
2	8	5	.64	.76	3.80
3	10	7	.80	1.10	7.70
4	12	9	−.14	−.14	1.26
5	8	5	.41	.44	2.20
6	12	9	.57	.65	5.85
7	12	9	−.11	−.11	.99
8	10	7	.75	.97	6.79
9	8	5	.46	.50	2.5
10	12	9	−.12	−.12	−1.08
11	12	9	.72	.91	8.19
Sum	*148*	*115*			*Sum 60.35* *Mean 0.52*
		Normal subjects following ACTH			
1	5	2	.68	.83	1.66
2	7	4	.89	1.42	5.68
3	10	7	.86	1.29	9.03
4	10	7	.74	.95	6.65
Sum		*20*			*Sum 23.02* *Mean 1.15*

N = Number of observations.
DF = Degrees of freedom.
r = Correlation coefficient.
z = Fisher's *z* transformation.

TABLE 5. CORRELATION COEFFICIENTS (*r* VALUE) FOR GROUP DATA OF A COLLEGE CREW, WITH AND WITHOUT STRESS PERIODS

Correlation	Total data		Stress excluded	
	r	Partial *r*	*r*	Partial *r*
17 KS *vs.* Uropepsin	+.163*	+.23*	+.41†	+.41†
17 OH *vs.* Uropepsin	−.07	−.17	−.07	−.12
17 OH *vs.* 17 KS	+.49†	+.51†	+.06	+.10

* Significant at 5% level.
† Significant at 1% level.

C. *Correlation coefficients on group data*

Correlation coefficients for group data on the 8 crew members are presented in Table 5. Both the r value and the partial r value were calculated (a) for the entire data, including control periods, time-trial and race; and (b) for the control periods alone, eliminating the stress periods of time-trial and race. The partial r values may be considered as indicating the relationship between any two variables, either independent of the third or when the third is held constant. In this way they may represent such a relationship more clearly.

A significant uropepsin-ketosteroid correlation was present, both for the total data (including the stress periods) and for the control periods alone. There was no significant correlation between hydroxycorticoids and uropepsin. The hydroxycorticoid-ketosteroid correlation was significant only for the total data; no significant correlation was evident when stress periods were excluded.

DISCUSSION

It is evident from the data presented that in the unstimulated normal male there is a varying pattern of adrenal-gastric correlation, with variation both between and within individuals in regard to the significance of the three correlations studied. Significant adrenal-gastric correlation occurs in the basal state more often than could be accounted for by chance alone; yet, in this basal state, significant correlations are of a low order of frequency and without a constant pattern. In contrast, in the subjects undergoing stimulation with corticotropin (ACTH), the correlation coefficients were generally high in the correlations tested for the period of corticotropin stress. Applying Fisher's z test, this difference between the corticotropin period and the control state in regard to the correlation coefficients for the uropepsin-ketosteroid relationship was noted to be of definite statistical significance.

These observations are in accordance with the "permissive" role of the adrenal cortex as described by Ingle (24, 25) and Engel (26, 27), and discussed in terms of "conditioning" factors by Selye (28).

We should be inclined to consider that in the basal state the stomach bears a semi-autonomous role relative to the adrenal cortex. However, under circumstances of corticotropin stimulation the stomach comes more directly under adrenal influences, as indicated by the correlations of the indices of gastric and adrenal activity.

Both Ingle and Engel believe that the adrenal hormone conditions the organism to respond to stress, whereas Selye contends that stress conditions the organism to respond to adrenal hormone. The present study,

wherein corticotropin without additional experimental stress produced a correlative metabolic response of both gastric and adrenal activity, would tend to support the former position.

Engel (9) has applied the permissive concept of adrenal-gastric activity to the role of the adrenal gland in the etiology of peptic ulceration. A full discussion of the etiologic factors in peptic ulcer disease is beyond the scope of this study. In the theoretical sense, however, we should agree with Engel that "in the presence of excess hormone, the gastric and duodenal mucosa might be thought of as being made more sensitive and responsive to those potentially noxious stimuli which in the absence of excess hormone would not be great enough to initiate ulceration." It should be emphasized, however, that although corticotropin may enhance the response of an organism to stress and thus predispose to ulceration, either corticotropin itself or the adrenal glucocorticoids can produce an increase in gastric acid, pepsin and uropepsin, as has been documented in man and in the experimental animal (3, 10).

Garst and Hilliard (29), using correlation coefficients to evaluate the uropepsin-ketosteroid relationship in 69 normal males ranging in age from 17 to 58 noted a significant relationship on their *group* data for the 20–40 age group. They did not measure or correlate the 17-hydroxycorticoids.

The correlations on group data of this present study support their observations in regard to a significant uropepsin-ketosteroid relationship (Table 5). The reason for this correlation is not clear, nor can we explain the absence of significant correlation between uropepsin and 17-hydroxycorticoids for this same controlled group. The significant group data correlation for the 17-hydroxycorticoids versus the 17-ketosteroids in the total data (including the stress periods), contrasted with the lack of correlation when stress periods were excluded, suggests that the increased adrenal output during the stress periods of time-trial and race accounted for the significant 17-hydroxycorticoid and 17-ketosteroid correlation present for the total group data. It should be noted, however, that there is a marked variation between and within individuals for any given correlation. Consideration of group data obscures this important individual variation.

SUMMARY

The relationship between gastric and adrenal activity in normal subjects before and after stimulation with corticotropin has been studied by means of statistical correlation coefficients. Uropepsin excretion was used as an index of gastric secretory function and adrenal urinary 17-hydroxycorticoid and 17-ketosteroid excretions were used as indices of adrenal activity.

1. In the basal state, there is marked variation between and within individuals in regard to correlative values for adrenal-gastric activity,

suggesting that under basal conditions the stomach acts semi-autonomously relative to the adrenal cortex.

2. Following stimulation with corticotropin, gastric peptic secretory activity was noted to come more directly under adrenal dominance.

3. These findings are discussed in terms of the "permissive" theory of hormonal activity.

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THE RELATIONSHIP OF ESTROGEN AND OF PITUITARY HORMONES TO THE METABOLIC EFFECTS OF PROGESTERONE*

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WHEN progesterone is administered to men and women in a dosage approximating the quantity secreted during early pregnancy (50–100 mg. daily), it induces a mild to moderate acceleration of protein catabolism (1–4) and an increased excretion of urinary sodium and chloride (1, 2). The enhanced catabolism appears to be a metabolic effect of progesterone itself, but the salt-dissipating influence can be explained on the basis of an inhibition of salt-retaining adrenal corticoids at a renal level. In subjects with normal adrenals, the catabolic process seems less intense and the increase in the excretion of urinary sodium is distinctly smaller than the same effects induced in hormone-treated Addisonians. Furthermore, only in individuals with intact adrenal glands is the sodium loss promptly recovered after progesterone treatment is discontinued. Since the natriuresis and chloruresis induced by progesterone seem to be dependent upon a competitive reaction, the presence of adrenal hormones is required for this response. Thus, the influence of progesterone on both protein and electrolyte metabolism is modified by the secretions of active adrenal glands.

No evidence was adduced in our original studies to suggest the participation of other glands of internal secretion in the evocation of the characteristic response to progesterone. Mediation of the enhanced catabolism by the thyroid gland seemed unlikely. The metabolic rates were not conclusively elevated during periods of increased nitrogen excretion, and urinary creatine and amino acid excretion were not elevated, as might have been anticipated during thyroid activation (5). None of the experiments reported was designed to elucidate a pituitary role not involving the adrenals. The response elicited in men demonstrated that ovarian secretions were not required. However, the fact that progesterone is normally se-

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creted after responsive tissues such as the uterus and breast have been prepared and sensitized by estrogen suggested that perhaps a similar relationship between estrogen and progesterone prevailed with regard to these general metabolic properties. In man, estradiol is both anabolic and salt-retaining when administered in large amounts; anabolism has also been demonstrated in association with dosages approaching the quantity secreted by normal ovaries (6–8).

The experiments to be reported here were designed to evaluate more extensively the relationship of the pituitary and thyroid glands and of estrogen to the metabolic expression of administered progesterone. In 2 women in whom ovarian function was absent or deficient, a comparison was made between the effects of progesterone given before and during a course of estrogen replacement. In a third study, the influence of progesterone was observed in an hypophysectomized, adrenalectomized, ovariectomized woman maintained with cortisone, desoxycorticosterone and thyroxine.

METHODS

All subjects were hospitalized on the metabolic unit and maintained on constant regimens in the usual manner (9). Daily 24-hour collections of urine were made and checked for completeness by means of creatinine determinations. The several urinary constituents were usually measured after making 48-hour pools, but on occasion 24-hour collections were used. Complete balance studies were not performed, but a relatively constant fecal excretion was assumed (10). A metabolic effect was defined as a significant systematic deviation from the control baseline of urinary excretion in association with treatment. An increased excretion was interpreted as a loss or catabolic effect, and a diminished excretion, as retention or anabolism. Total urinary nitrogen, inorganic phosphorus, creatine, creatinine and chloride were determined as noted in a previous report (9). The estimation of urinary sodium and potassium was performed with the Perkin-Elmer flame photometer, using lithium as an internal standard.

The progesterone and estradiol benzoate[1] used in these studies fulfilled U.S.P. criteria for purity. Both were administered by intramuscular injection, as were the cortisone acetate and desoxycorticosterone acetate in 1 subject. The replacement dosage of estradiol which was chosen (0.5 mg. daily) was assumed to approach the average amount of estrogen secreted by the normal ovary. Allen (10) reported that the equivalent of 0.3 mg. of estradiol benzoate was the average minimum of estrogen required to induce withdrawal bleeding in postmenopausal women, and Papanicolaou and Shorr (11) found that 0.2 to 0.3 mg. per day was the minimum required to produce vaginal cornification in most hypogonadal women. The dosage of progesterone used in most of our previous studies was 50 mg. per day; for comparative purposes, the same quantity was given in these experiments.

PATIENTS

A. M. (Fig. 1) was a 52-year-old housewife with moderately severe rheumatoid arthritis of six years' duration. Occasionally, her temperature was slightly elevated. The

[1] Progesterone and estradiol benzoate were generously supplied by Dr. Edward Henderson of Schering Corporation, Bloomfield, N. J.

menopause had occurred five years before this hospitalization. Throughout the course of the investigation, she received 0.6 Gm. of acetylsalicyclic acid four times a day; on rare occasions, toward the end of the study, she required 0.06 Gm. of codeine sulfate. She experienced remissions and exacerbations of the rheumatoid process, but none of these could be correlated with the beginning or the discontinuation of either progesterone or estradiol benzoate treatment. Her metabolic rate was −18 per cent (Mayo Foundation standard) with a basal caloric requirement of 1,110 per twenty-four hours.

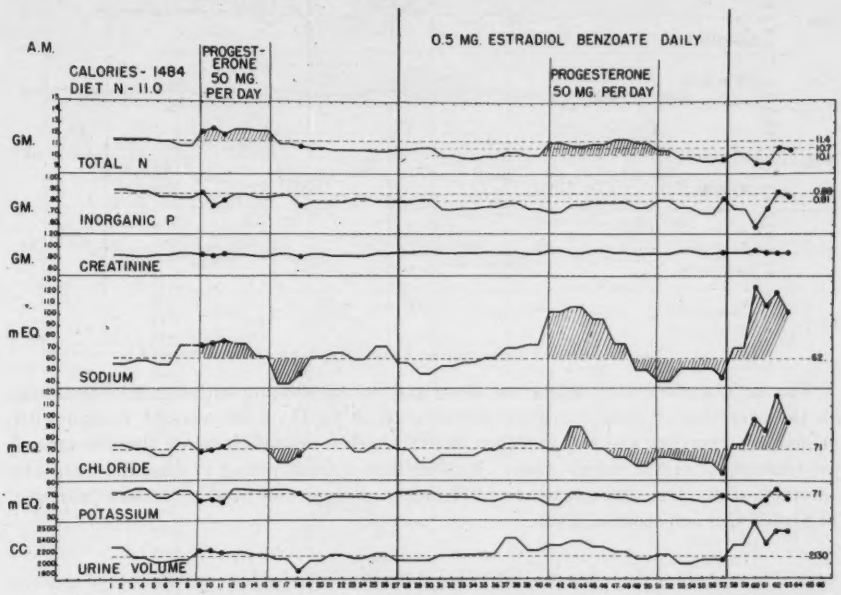

FIG. 1. The effect of progesterone alone and in combination with estradiol benzoate on the excretion of several urinary constituents in A. M., a 52-year-old postmenopausal woman. Broken horizontal lines indicate the averages of pre-treatment control values. Heavy vertical lines delimit the period of treatment with estradiol benzoate. The diet consisted of 174 Gm. carbohydrate; 69 Gm. protein (nitrogen 11.0); 58 Gm. fat; calories, 1,484.

G. G. (Fig. 2) was a 30-year-old woman. She was 4 feet 5 inches tall, and had a webbed neck and an increased carrying angle at the elbow. Axillary hair was absent; pubic hair was sparse. The external and internal genitalia were infantile; no breast tissue could be identified, and the nipples were small and widely spaced. She had never menstruated. The metabolic rate was −2 per cent with basal calories of 1,030 per day.

M. R. (Fig. 3) was a 50-year-old woman with metastatic carcinoma of the breast. Despite this serious ailment, she was ambulatory and in good general condition. Two years previously, she had undergone bilateral oophorectomy and bilateral adrenalectomy. After an initial improvement in the metastatic bone lesions, there was evidence of recurrent growth. Shortly before these studies were performed, the pituitary gland had been destroyed by radioactive yttrium beads implanted by Dr. Eric Youle. Recovery

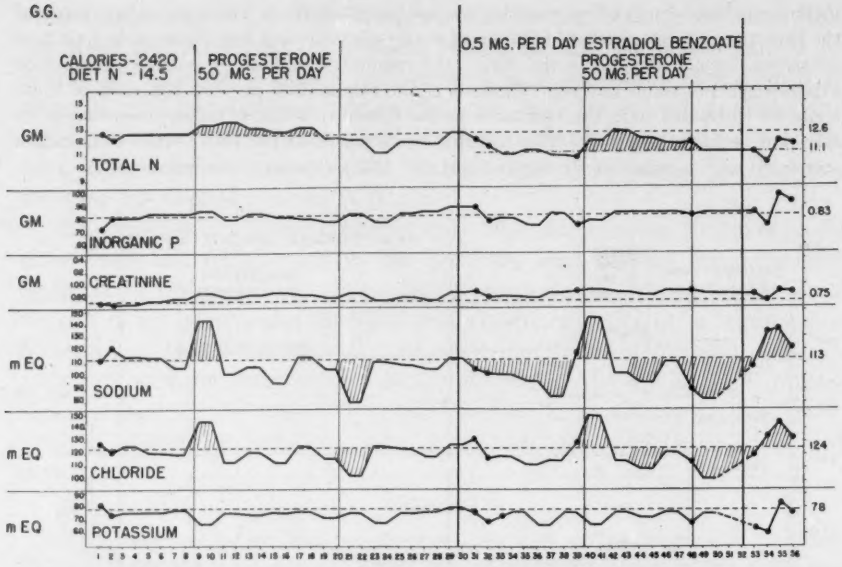

FIG. 2. The effect of progesterone alone and in combination with estradiol benzoate on the excretion of several urinary constituents in G. G., a 30-year-old woman with primary amenorrhea and short stature. Broken horizontal lines indicate the averages of pre-treatment control values. Heavy vertical lines delimit period of estradiol benzoate administration. The diet consisted of 217 Gm. carbohydrate; 91 Gm. protein (nitrogen 14.5); 132 Gm fat; calories, 2,420.

from this operation was uneventful. Fifteen days after the hypophysectomy, sodium *l*-thyroxine was started in a dosage of 0.15 mg. daily. Despite the fact that she was receiving 50 mg. of cortisone acetate and 1 mg. of desoxycorticosterone acetate intramuscularly each day, this rapid introduction of thyroid hormone induced a moderately severe Addisonian crisis. She recovered promptly from the acute adrenal insufficiency, and the foregoing maintenance therapy was continued. The metabolic rate was −19 per cent, with basal calories of 990 per day.

RESULTS

Progesterone was catabolic in the hypo-ovarian women, whether it was administered before or during treatment with estradiol benzoate. The results observed were characteristic of the metabolic effects of progesterone in subjects with intact adrenals, in that the enhancement of protein catabolism was expressed solely by an elevation in the urinary nitrogen output. Urinary inorganic phosphorus excretion was not affected (2). The elevation in urinary nitrogen was of the same order of magnitude and of approximately the same duration relative to the length of treatment when

the progesterone was given before the introduction of estrogen replacement as it was after the estradiol effect was established.

Estradiol benzoate in a dosage of 0.5 mg. per day was anabolic in both subjects. In A. M. (Fig. 1) a new baseline, 0.7 Gm. lower than the initial

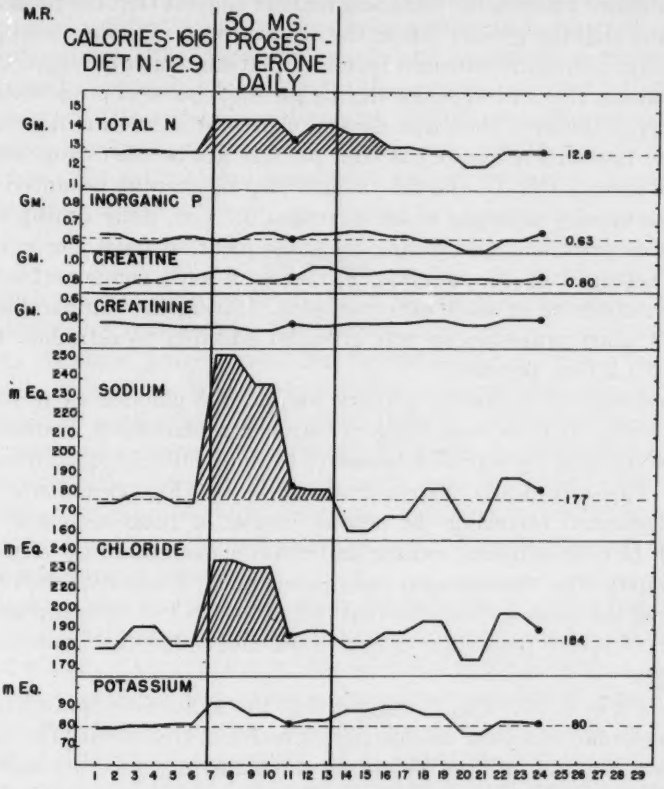

FIG. 3. The effect of progesterone on the excretion of several urinary constituents in an hypophysectomized, adrenalectomized, ovariectomized woman aged 50. Broken horizontal lines indicate the averages of pretreatment control values. The diet consisted of 115 Gm. carbohydrate; 73 Gm. protein (nitrogen 11.7); 96 Gm. fat; calories, 1,616.

controls, was assumed after the first course of progesterone. As a result of treatment with estrogen, the urinary nitrogen level then dropped another 0.6 Gm. In G. G. (Fig. 2) estradiol administration was followed by a decline in nitrogen excretion of about 1.5 Gm. Except for the intervals of progesterone treatment, urinary nitrogen was suppressed for the duration of estrogen treatment in both experiments (twenty-nine days in A. M., and

twenty-two days in G. G.) and rose again three or four days after estradiol was discontinued. As a result of this anabolic response, it was necessary to measure the rise in urinary nitrogen induced by progesterone from different points of reference in the two circumstances in which it was given.

Calculations made in the foregoing manner suggest that the progesterone effect was slightly greater when the subject was receiving estrogen. In A. M. (Fig. 1) urinary nitrogen rose to a plateau which averaged 0.8 Gm. higher than in the control period during the first course of progesterone. After estradiol therapy, the same dosage of progesterone caused urinary nitrogen to rise an average of 1.2 Gm. per day above the estrogen-induced anabolic level. In G. G. the first course of progesterone produced an elevation in urinary nitrogen which averaged 0.7 Gm. daily during the first six days of treatment, but during the subsequent six days, the level of excretion returned to the baseline. Evidence of such compensatory forces has been observed in some other subjects. During the comparable initial six days when progesterone was given in addition to estradiol, the rise averaged 1.2 Gm. per day.

Progesterone also affected urinary sodium and chloride excretion in its characteristic manner, regardless of estrogen replacement. Sodium retention was induced by estradiol benzoate in the relatively small dosage employed. This was clearly demonstrated in G. G. (Fig. 2) and was suggestively indicated following the second course of progesterone in A. M. (Fig. 1). In both subjects, sodium and chloride excretion rose very sharply immediately after the estrogen was discontinued. These rebounds strongly supported the other indications that salt excretion had been suppressed by estradiol. Urinary potassium excretion was not influenced by either of the hormones.

The data in A. M. (Fig. 1) suggest that the sodium-dissipating influence of progesterone was more intense during estrogen treatment. The elevation in urinary sodium induced before estradiol therapy was unusually slight and would probably be regarded as insignificant in view of the rather wide spontaneous variations in urinary sodium, were it not for the uniformity of this response to progesterone. The sodium loss appeared substantially greater during estrogen treatment, but this could have been partially artifactual, in that there was an apparent rise in the sodium baseline prior to the administration of progesterone. In G. G. the sodium and chloride losses and the post-treatment recoveries of these ions were almost superimposable in the two situations. Thus it cannot be concluded from these two experiments that estradiol enhances the sodium-dissipating influence of progesterone.

The effects of progesterone in the hypophysectomized, adrenalectomized, ovariectomized woman receiving adrenal and thyroid replacement therapy

(Fig. 3) were similar to those previously observed in treated Addisonians. The tendency for the catabolic process to be more intense in patients with Addison's disease than in subjects with normal adrenals was not so distinct in this instance. The elevation of urinary nitrogen averaged only 1.5 Gm. per day and was not unusually protracted. However, there were suggestions of parallel elevations in urinary potassium, creatine and inorganic phosphorus, the potassium rise alone approaching significance. Progesterone has been shown to increase the excretion of these constituents only in patients with adrenal insufficiency. Sodium and chloride excretion rose dramatically the first four days of treatment (sodium loss, 277 mEq.; chloride loss, 204 mEq.). Post-treatment sodium retention, if any, was relatively slight.

DISCUSSION

Judging from the study on M. R. (Fig. 3), the pituitary gland and its secretions are not required for the induction of the catabolic and salt-dissipating effects of progesterone. No hypophyseal principle is known to be catabolic in itself, so that the possibility that progesterone promoted the secretion of a pituitary agent which directly accelerated protein catabolism was never seriously considered. However, inhibition of an anabolic secretion such as growth hormone could conceivably have accounted for this effect, or a pituitary hormone could have participated in the process in a permissive fashion. The full response in M. R. indicates that neither of these conjectures is correct.

Furthermore, the results of this study support the previous indications that the catabolic influence of progesterone is not mediated by the thyroid or adrenal glands. The response in this adrenalectomized subject (M. R.) confirms the impression of adrenal independence previously gained from the studies in Addison's disease. The adrenal crisis provoked by the introduction of thyroxine (*see* protocol, M. R.) suggests that the anticipated secondary atrophy of the thyroid and hypothyroidism had developed by this time. Accordingly, the possibility that the thyroid gland was activated twenty-one days later when progesterone was administered seems remote. No studies have been carried out in the absence of thyroid hormone, so it cannot be said that its presence is not required.

Although some quantitative differences were observed when the effects of progesterone administered before estrogen were compared with the effects after estrogen, their experimental significance is questionable. The apparent enhancement in the elevation of urinary nitrogen when progesterone was administered during estrogen treatment amounted to 0.4 Gm. in Subject A. M., and 0.5 Gm. in Subject G. G. Considering the sensitivity of the method and the possible variables introduced by means of calcula-

tion and the limited number of experiments, these differences hardly denote a potentiation by estrogen. In our other studies there was a tendency for progesterone to be more catabolic when nitrogen balance was positive. If the slight differences observed here were to be confirmed by repetition of these experiments, the enhancement of the catabolic process might be related to the anabolic state and not specifically to the presence of physiologic amounts of estrogen.

In both experiments, the anabolism induced by estrogen replacement protected the organism from the catabolic influence of progesterone without in any way interfering with its characteristic effect. A negative nitrogen balance was avoided or minimized. It should be appreciated, however, that in the natural course of events, endogenous progesterone is seldom if ever secreted within so short an interval after the initial secretion of estrogen. The nitrogen retention induced by estrogen doubtlessly wanes in time, and if administered during nitrogen equilibrium, progesterone would be expected from these data to induce a negative nitrogen balance despite the presence of estrogen. This has been shown to be the case in 2 women whose ovaries were secreting estrogen when progesterone was administered (2). In the normal menstruating woman and in pregnancy, acute increments in estrogen secretion which approach the amounts given here might be expected to be anabolic and thus conceal or negate the effects of progesterone on protein metabolism.

The demonstration that an approximately physiologic quantity of estradiol benzoate may induce salt retention supports the general consensus that estrogen may exert an important influence on shifts in electrolytes during the menstrual cycle as well as in pregnancy. There was no indication that estradiol counteracted the sodium-dissipating effect of progesterone. If this influence of progesterone was affected at all, it was enhanced in the presence of estrogen. Had this enhancement been convincing, one could speculate that progesterone inhibits the sodium-retaining effect of estrogen, as it appears to inhibit that of corticoids.

SUMMARY AND CONCLUSIONS

A study has been made of the effect of pituitary hormones and of estrogen on the metabolic pattern following the administration of progesterone.

1. Progesterone was catabolic and intensely sodium and chloride-dissipating in an hypophysectomized, adrenalectomized, ovariectomized woman maintained with thyroxine, cortisone and desoxycorticosterone. These effects were not mediated by the pituitary, adrenal or thyroid glands, and did not require the presence of hypophyseal secretions.

2. Approximately a replacement quantity of estradiol benzoate (0.5 mg. daily) was mildly anabolic and salt-retaining in 2 women with ovarian deficiency.

3. A comparison of the effects of progesterone before and during estradiol treatment in these 2 women produced no conclusive evidence that the catabolic influence of progesterone was potentiated by estrogen. There was also no direct antagonism, but the anabolism induced by estradiol prevented a significant net loss of nitrogen when progesterone acted catabolically.

4. The sodium-dissipating effect of progesterone was probably not materially influenced by the administration of 0.5 mg. of estradiol benzoate daily.

5. The anabolic and sodium-retaining properties of estradiol must be taken into account when considering the influence of progesterone on protein and electrolyte metabolism in normal women and in pregnancy.

Acknowledgment

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METABOLIC EFFECTS OF A SINGLE INTRAVENOUS INFUSION OF HYDROCORTISONE RELATED TO PLASMA LEVELS IN A NORMAL VERSUS AN ADRENALLY INSUFFICIENT SUBJECT*

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ALTHOUGH numerous studies of the metabolic effects of adrenal steroids in man have been made, little information is available regarding the time of onset and duration of effect following their administration as a single dose to either normal subjects or patients with adrenal insufficiency. Fourman and his associates (1) reported metabolic studies following a single intramuscular injection of 30 mg. of desoxycorticosterone acetate in oil into a normal subject. This group also determined the metabolic effects of 50 mg. of hydrocortisone administered intramuscularly to a normal male in four doses over three hours (2). Others (3–5) have studied the metabolic changes induced by cortisone, corticosterone, or hydrocortisone administered by continuous intravenous infusion for periods of four to eight hours at rates of 1 mg. to 25 mg. per hour, but the duration of effect was not reported. Most metabolic experiments have involved the use of repeated injections of steroids for several days, with collection periods varying from one to six days.

Since hydrocortisone has been identified as an important component of human adrenocortical hormone, its concentration in adrenal venous blood being higher than that of any other steroid (6, 7), a study was undertaken

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to determine the magnitude and duration of the metabolic effects following a single relatively rapid intravenous infusion of 100 mg. of this steroid. The availability of a technic for quantitatively measuring its level in the blood (8, 9) enabled a correlation of blood levels with metabolic changes. In order to compare the effects obtained when the body contained normal quantities of hydrocortisone with those observed when the tissues were depleted of this steroid, 2 subjects were employed—one with normal adrenal function and the other having adrenal insufficiency. The latter patient was studied twice, once while he was being maintained in a state of eucorticism, and again forty-eight hours after withdrawal of maintenance hydrocortisone therapy.

EXPERIMENTAL DESIGN AND METHODS

The 2 volunteer subjects were of the same race and sex and approximately the same age, weight, and body build. One (B.R.) was a 44-year-old colored man who was normal except for an asymptomatic persistent left superior vena cava and possible congenital pulmonic stenosis. The other (W.J.) was a 46-year-old Negro with adrenal insufficiency associated with chronic pulmonary tuberculosis. His disease had been satisfactorily controlled and his condition had been stable for two weeks on a maintenance regimen of 10 mg. of hydrocortisone orally every twelve hours, 3 Gm. of para-aminosalicylic acid three times daily after meals, 1 Gm. of streptomycin twice weekly, and a single 75-mg. pellet of desoxycorticosterone acetate implanted subcutaneously.

Both subjects were kept on the metabolic ward for the duration of the study. Each received an identical diet containing 19.4 Gm. of nitrogen, 200 mEq. of sodium, 116 mEq. of potassium, and 2200 calories daily, and an identical fluid intake of 2500 ml. per day. The daily intake was divided so that half was administered in the 8-hour period between 7 A.M. and 3 P.M., and half in the 8-hour period between 3 P.M. and 11 P.M. There was no intake between 11 P.M. and 7 A.M. Such a regimen enabled comparison between corresponding 8-hour periods of each day. Patient W.J.'s oral hydrocortisone schedule was changed to 5 mg. every six hours to provide a more constant intake throughout each 24-hour period. Para-aminosalicyclic acid was continued, but streptomycin was withheld during the study.

After three days on this regimen to allow for equilibration, control urine samples were collected over the three 8-hour periods for base-line determinations. The subjects were then given 100 mg. of hydrocortisone[1] in 500 ml. of 5 per cent dextrose solution containing 2 per cent ethanol, by intravenous infusion over a 30-minute period beginning at 7 A.M. Urine collections in 8-hour periods continued for forty-eight hours after the infusion. W.J. received his last 5-mg. dose of hydrocortisone orally three hours before the infusion, and no further hydrocortisone was given during the forty-eight hours following the infusion. Blood samples were drawn for plasma hydrocortisone determinations before and $\frac{1}{4}$, $\frac{1}{2}$, 1, $1\frac{1}{2}$, $2\frac{1}{2}$, $4\frac{1}{2}$, $8\frac{1}{2}$, 16, 24 and 48 hours after the beginning of the infusion. Following this study, when W.J. had received no hydrocortisone for forty-eight hours, another infusion was administered and determinations were repeated in an identical manner over the next forty-eight hours. The presence of the DCA pellet should not have interfered with the study, since this steroid would not influence determinations

[1] The hydrocortisone used in this study was generously supplied by Dr. Elmer Alpert of the Merck Laboratories.

of hydrocortisone, and since its relatively small sodium-retaining effect was assumed to be constant throughout the entire period of observation.

Plasma levels of hydrocortisone were determined by the fluorescent method of Sweat (8, 9). Urine samples were analyzed for free and conjugated 17,21-dihydroxy-20-ketones by the method of Glenn and Nelson (10). Eosinophils were counted by Pilot's technic (11) Sodium and potassium were determined by flame photometry utilizing a lithium internal standard; nitrogen, by the macro-Kjeldahl technic; and creatinine, by a modification of the Folin-Wu method (12). Blood samples for creatinine determinations were drawn at suitable intervals to enable calculations of creatinine clearances for each 8-hour period.

As an additional control for this study, another normal volunteer subject entered the metabolic ward under an identical diet and metabolic schedule. After a period of equilibration, urine collections were made for twenty-four hours without any infusion; then for twenty-four hours after an infusion of 500 ml. of 5 per cent dextrose solution over thirty minutes; then for forty-eight hours after an infusion of 500 ml. of 5 per cent dextrose containing 2 per cent ethanol, over the same period. This study was made in order to determine the effect of an infusion of dextrose alone and of an infusion of dextrose containing ethanol in the concentration used in the administration of hydrocortisone in the other 2 subjects. The same determinations were carried out as in the other studies, except that blood and urine hydrocortisone levels were not assayed.

RESULTS

Effect of the Vehicle

The infusions of 500 ml. of 5 per cent dextrose solution alone and of 500 ml. of 5 per cent dextrose containing 2 per cent ethanol into the normal subject over a period of thirty minutes were followed by relatively slight increases in water excretion over the ensuing 8-hour periods as compared with corresponding control periods (58 ml. and 111 ml., respectively). Hence any diureses which might have occurred were largely obscured by the length of the collection periods. Furthermore, the variations in excretion of sodium, potassium, creatinine and nitrogen bore no resemblance to the pattern to be expected with water diuresis or alcohol ingestion (13), or to those observed following the administration of hydrocortisone. It therefore appears unlikely that the vehicle in which the hydrocortisone was administered significantly affected the results described.

Effect of Hydrocortisone

Normal subject

The data obtained during the control day and during the forty-eight hours following administration of hydrocortisone to the normal subject are listed in Table 1. In Figure 1 these data are plotted to show the difference between values before and after the infusion during comparable periods. The urinary excretions during each period of the control day serve as the zero base line, and differences between comparable periods on the two

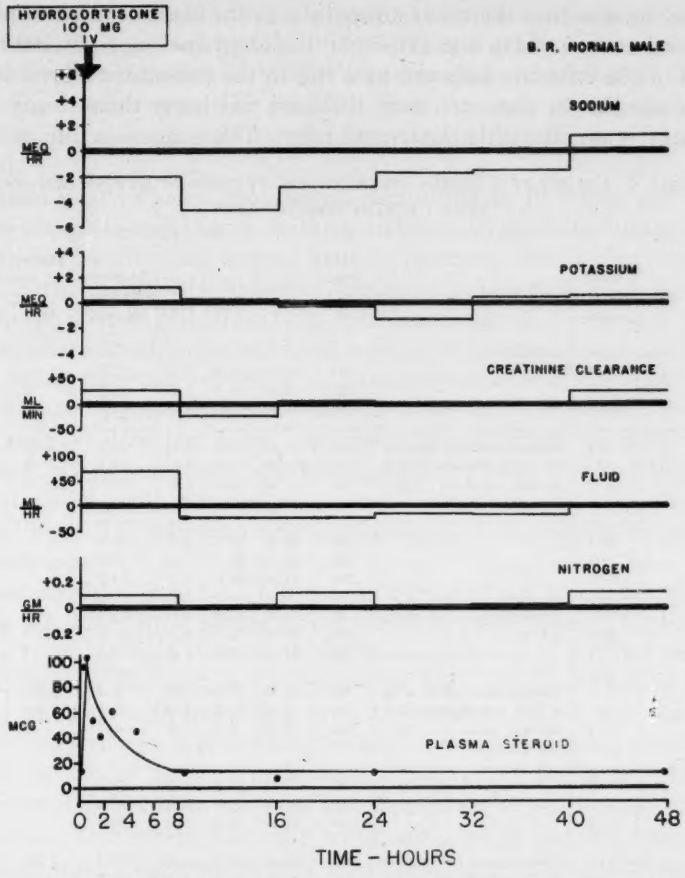

FIG. 1. Effect of a single intravenous infusion of hydrocortisone upon urinary constituents and plasma hydrocortisone levels in the normal subject. The changes in urinary constituents are plotted relative to their excretions during corresponding control periods, which serve as the base line. Retention relative to the control period is indicated by the area enclosed below the base line, and loss relative to the control period by the area enclosed above the base line. The nitrogen scale is 6.7 times as great as it would be were it plotted in relation to potassium in the proportion in which they occur in protoplasm (20).

days following the infusion are plotted in such a manner that the effect of hydrocortisone is presented directly, without the confusing factors of diurnal variation in urinary excretion and lack of intake during the overnight periods. In order to facilitate comparison, plasma hydrocortisone levels are plotted below the metabolic data.

It may be seen that there was a rapid rise in the concentration of plasma hydrocortisone, reaching a peak of 101.9 micrograms (μg.) per 100 ml. at the end of the infusion, followed by a fall to the pre-infusion level by the eighth hour. At the sixteenth hour the level was lower than at any other time, but it was still within the normal range. This suggests a mild rebound

TABLE 1. EFFECT OF A SINGLE INTRAVENOUS INFUSION OF HYDROCORTISONE UPON URINARY CONSTITUENTS

Subject	Day	Period (specific hours)	Therapy	Urine vol. (ml.)	Na (mEq.)	K (mEq.)	N (Gm.)	Creatinine clearance (ml./min.)	Urinary-17-OH-steroids*	
									Free (μg.)	Conjugated (μg.)
B.R. (normal male)	1	7- 3		425	43.36	36.96	4.23	92.4	0	4,032
		3-11		525	59.36	35.68	5.35	99.8		
		11- 7		537	58.56	17.20	4.07	83.4		
	2	7- 3	Hydrocortisone, 100 mg. in 500 ml. 5% dextrose/water (2% ethanol), given i.v., 7:00-7:30 A.M.	972	27.12	47.00	5.11	119.5	3,610	19,309
		3-11		345	21.36	38.32	5.51	76.7	48	4,512
		11- 7		360	36.00	15.12	5.02	89.1	48	1,392
	3	7- 3		300	30.00	26.72	4.34	88.6	0	187
		3-11		398	47.76	39.84	5.55	98.6		
		11- 7		530	68.88	22.24	5.14	112.0		
W.J. (adrenal insufficiency)	1	7- 3	Hydrocortisone 5 mg. orally, q. 6 hrs.	450	77.81	34.64	4.13	78.1	158.4	3,124
		3-11		400	68.40	34.40	4.45	63.9		
		11- 7		780	71.76	23.80	4.36	63.0		
	2	7- 3	Hydrocortisone, 100 mg. in 500 ml. 5% dextrose/water (2% ethanol), given i.v., 7:00-7:30 A.M.	605	42.00	42.28	2.33	76.4	6,969	20,150
		3-11		268	19.60	24.62	3.94	53.9	768	1,014
		11- 7		530	29.68	6.88	4.03	40.5	0	0
	3	7- 3		467	67.68	11.20	6.16	50.3	0	0
		3-11		688	130.72	29.60	5.52	83.1		
		11- 7		570	87.20	18.80	5.21	69.2		
	4	7- 3	Hydrocortisone, 100 mg. in 500 ml. 5% dextrose/water (2% ethanol), given i.v., 7:00-7:30 A.M.	1845	53.56	48.28	4.80	242.4	3,204	27,126
		3-11		282	14.96	11.84	4.89	60.9	33.6	720
		11- 7		330	30.00	9.28	4.23	76.4	0	0
	5	7- 3		395	68.32	11.04	5.35	83.7	0	0
		3-11		615	123.00	33.20	5.60	116.7		
		11- 7		525	95.52	22.96	7.09	102.5		

* 17.21-Dihydroxy-20-ketones.

effect due to inhibition of endogenous corticotropin, but the change was not sufficiently great to be significant.

Sodium retention was striking, beginning during the first eight hours after infusion and being maximal between the eighth and sixteenth hours. It persisted until the fortieth hour, followed by a slight loss, probably representing an incipient rebound sodium diuresis. A total relative retention of 101.76 mEq. of sodium occurred during the 40-hour period. It will

be noted that the early increase in sodium excretion following hydrocortisone infusion described by others (3, 5, 14) and attributed to an increase in glomerular filtration rate (GFR) was not apparent here. Such a change may have occurred within the first hour or two, subsequently to be obscured by ensuing sodium retention for the remainder of the 8-hour period.

A mild relative loss of potassium, amounting to 10 mEq., was evident during the initial eight hours, with an additional 2.64 mEq. during the next period, but by the thirty-second hour the quantity lost had been regained.

Endogenous creatinine clearance ranged from 83.4 to 99.8 ml. per minute during the control 24-hour period, and this increased 29 per cent to 119.5 ml. per minute during the first eight hours after infusion. Since this amount of variation was observed following the administration of the vehicle alone, and since endogenous creatinine clearance does not reflect GFR in man as accurately as does the inulin clearance (15), it cannot be definitely attributed to hydrocortisone effect in this study; but a rise in GFR of this magnitude has been reported in normal human subjects (14, 16) and in normal dogs (17) following administration of corticotropin, cortisone, or hydrocortisone.

Fluid excretion increased 130 per cent, from 53 ml. to 122 ml. per hour, during the same initial 8-hour period following the infusion. This was probably a result of both increase in GFR and decrease in tubular reabsorption of water. Burston and Garrod (18) have reported that in normal human subjects with urine flow rates of less than 1.5 ml. per minute (90 ml. per hour) there is a direct relationship between urine flow and creatinine output. At faster rates of urine flow, urine output is related chiefly to tubular reabsorption of water—a function which depends upon the level of pituitary antidiuretic hormone, which enhances it, and of adrenocortical hormone, which depresses it (19). Since creatinine output did increase in this subject, part of the increased fluid excretion was probably secondary to a rise in GFR; but since the rate of urine output reached 122 ml. per hour, a portion of the increase must have been due to decreased tubular reabsorption as well. Following the initial diuresis, water was retained between the eighth and fortieth hours, totalling 609 ml. During this same interval the amount of sodium retained was 85.5 mEq., yielding a proportion of 140 mEq. of sodium per liter of fluid, and suggesting that sodium and water were retained in isotonic proportion.

The changes in nitrogen excretion demonstrated a different pattern. A mild loss occurred in the first eight hours, and this persisted in varying degree throughout the forty-eight hours of observation, totalling 3.31 Gm. Fourman, *et al.* (2) observed a similar phenomenon following the intramuscular injection of 50 mg. of hydrocortisone, with a cumulative loss of

approximately 1.6 Gm. over forty-eight hours before a beginning retention developed. Also the nitrogen excretion in the first eight hours of this study, as in the early stages of Fourman's study, was not sufficient to explain the potassium loss on the basis of breakdown of protoplasm (20). It is possible that, following protoplasmic breakdown, potassium may be excreted more rapidly than nitrogen. After the first period, the tendency to lose nitrogen was greater during the 11 P.M.–7 A.M. periods on both days, suggesting that this tendency is greater during periods of fasting or sleep.

Circulating eosinophils fell from 134 per cu. mm. before infusion to 11 per cu. mm. four hours after infusion, and were still at this level at the eighth hour. Twenty-four hours after infusion the count had returned to 153 per cu. mm. No other eosinophil counts were made, so the precise duration of the eosinopenia cannot be stated.

A detailed consideration of the urinary steroid excretion has been reported separately (21, 22), but it is interesting to note in Table 1 that the excretion of conjugated 17,21-dihydroxy-20-ketones had returned to pre-infusion levels after the sixteenth hour, although a small quantity of free steroid was excreted up to the twenty-fourth hour. The low excretion of steroid from the twenty-fourth to the forty-eighth hour suggests a rebound effect.

Adrenal insufficiency

The results of both studies on the patient with Addison's disease are presented in Table 1 and Figure 2. As with the normal subject, the concentration of plasma hydrocortisone reached its peak at the end of the infusion, attaining levels of 131.8 μg. per 100 ml. in the study started during therapy, and 165 μg. per 100 ml. following the infusion begun forty-eight hours after the last dose of hydrocortisone. Whether these levels are significantly different from each other, or from the peak of 101.9 μg. per 100 ml. reached in the normal subject, cannot be definitely stated. The fall in plasma level was again rapid, returning to normal range by the eighth hour in the study after maintenance therapy and by the sixteenth hour in the withdrawal study. There was a further fall to very low levels in both instances, and these subnormal levels persisted throughout the remainder of each 48-hour period.

The pattern of change in sodium excretion was practically identical following both infusions. Retention was evident during the first eight hours, maximal during the second eight hours, and persisted till the thirty-second hour, followed by a relatively marked sodium diuresis. The cumulative retentions in the first thirty-two hours were 131.88 mEq. and 129.0 mEq., respectively, and the losses from the thirty-second to the forty-eighth hour were 77.76 mEq. and 78.36 mEq., respectively. The sodium

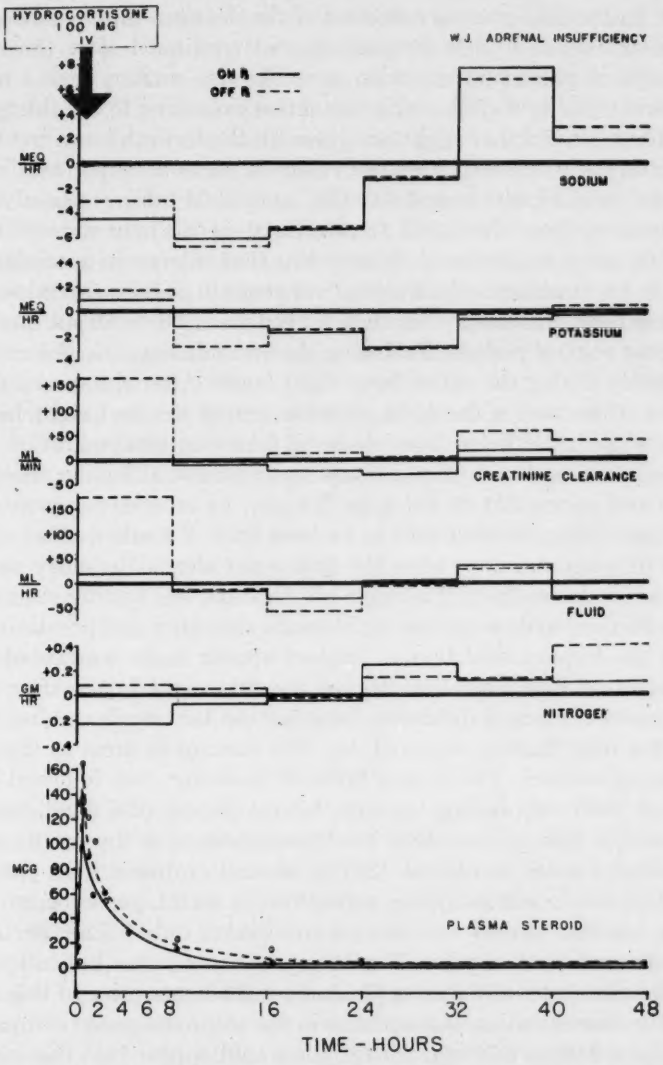

FIG. 2. Effect of a single intravenous infusion of hydrocortisone in a patient with adrenal insufficiency while receiving maintenance hydrocortisone therapy (solid line) and forty-eight hours after cessation of therapy (interrupted line). The changes are plotted as in Figure 1.

diuresis occurred earlier and was more pronounced than that seen in the normal, and probably was a reflection of the deficient adrenocortical state, since maintenance hydrocortisone was not continued after the infusion.

Changes in potassium excretion were likewise similar, with a mild loss in the first eight hours followed by retention extending to the thirty-second hour. There was further slight retention till the fortieth hour, but this was not significantly different from the values in the control period. The losses in the first eight hours were 7.64 mEq. and 13.64 mEq., respectively, and the retentions from the eighth to the thirty-second hour were 47.14 mEq. and 57.68 mEq., respectively. It is evident that changes in potassium excretion bore no consistent relationship to changes in sodium excretion.

Endogenous creatinine clearance ranged from 60 to 80 ml. per minute during the control periods. Following the first infusion, this did not change remarkably during the entire forty-eight hours. After the second infusion, however, there was a threefold increase during the first eight hours. Although a rise in GFR has been observed following administration of corticotropin, cortisone, or hydrocortisone to hypoadrenal human subjects (14, 16, 18) and adrenalectomized dogs (23, 24), no increases approached this magnitude. Why there should have been such a marked effect after the second infusion and none after the first is not clear. Obviously no conclusions can be drawn from this single observation, but further studies of the acute effects of hydrocortisone upon inulin clearance and creatinine excretion in the hypoadrenal human subject appear to be warranted.

Changes in fluid excretion during the first eight hours after infusion also showed a marked difference between the two studies. After the first infusion a mild diuresis occurred, but this was not as great as that seen in the normal subject. The second infusion, however, was followed by a diuresis of 1845 ml. during the first 8-hour period (230.6 ml. per hour). A diuresis of this magnitude is not inconsistent with the results observed by Garrod, Davies and Cahill (24) in adrenalectomized dogs given intravenous cortisone acetate plus a water load of 40 ml. per kilogram of body weight, but this subject received a water load of only 7.5 ml. per Kg. with his hydrocortisone infusion. Furthermore, there is no indication of significant water retention during the forty-eight hours prior to this infusion. Since the concentration of creatinine in the urine decreased comparatively little (from 0.92 to 0.74 mg. per ml.), it would appear that this increase in fluid excretion was largely due to an increase in GFR. Beyond the eighth hour after infusion, fluid retention was practically identical in both studies. It is interesting to note that sodium retention occurred during the initial eight hours in spite of the water diuresis, and also that sodium was subsequently retained in excess of water in both studies.

After the first infusion, relative nitrogen retention occurred during the

initial twenty-four hours (totalling 2.64 Gm.) followed by a loss (3.94 Gm.) over the next twenty-four hours. With the second infusion a small relative nitrogen loss occurred during the first sixteen hours, succeeded by a slight retention before a more rapid loss ensued from the twenty-fourth to the forty-eighth hour. The relative loss in the first twenty-four hours amounted to 0.98 Gm., and in the second twenty-four hours to 5.92 Gm., totalling 6.89 Gm. for the study. The increased nitrogen excretion during the second twenty-four hours after infusion in both studies was comparable, and may have been a late effect of hydrocortisone administration, but the reason for the discrepancy in the behavior during the first sixteen hours is not clear. Possibly the relative nitrogen diuresis at the end of the first study affected the response to the second infusion. It is apparent that changes in nitrogen excretion were not correlated with changes in urinary output of potassium, creatinine or fluid.

The circulating eosinophil count fell from an initial level of 66 to 2 per cu. mm. four hours after the first infusion and to zero at the eighth hour. By the twenty-fourth hour the count had returned to 165, and at the forty-eighth hour (just prior to the second infusion) to 140 per cu. mm. After the second infusion it fell to 12 per cu. mm. at four hours and to zero at eight hours, but it had returned to 271 at twenty-four hours and to 390 per cu. mm. at forty-eight hours. Since eosinophil counts were not made between the eighth and twenty-fourth hours, the exact duration of the depression is not evident.

Urinary steroid excretion (Table 1) exhibited a relationship to plasma levels, disappearing after these levels fell below normal.

DISCUSSION

The metabolic changes observed following the single intravenous infusion of 100 mg. of hydrocortisone over a 30-minute period into the normal subject in this study were in general similar to those observed by Fourman and his associates (2) following the intramuscular injection of 50 mg. of this steroid in four doses over a 3-hour period, namely, sodium retention, potassium loss and nitrogen loss. The degree of sodium retention and nitrogen loss was somewhat greater, as would be expected, with the larger dose, but potassium loss was less in amount and shorter in duration than that observed in the earlier study. This may have been related to the difference in mode of administration of the steroid.

The metabolic effects in the patient with adrenal insufficiency, following the first infusion given at a time when his tissues presumably contained normal quantities of hydrocortisone, were somewhat different from those observed in the normal subject. These differences apparently resulted in part from the development of adrenal insufficiency after the effects of the

infusion had subsided. Sodium retention did not last as long and rebound sodium diuresis was greater, as would be expected in the adrenal-deficient state. In both subjects, during the first eight hours after infusion, sodium retention occurred in spite of increased water excretion. Subsequently, the normal subject retained sodium and water in isotonic proportion, whereas the Addisonian patient continued to retain sodium in excess of water. Also after the eighth hour, potassium retention was greater in the Addisonian patient. The initial nitrogen retention in this man was in contrast to the loss observed in the normal subject.

There was remarkably little difference between the effects upon sodium or potassium excretion in the Addisonian patient following the second infusion administered at a time when his tissues were relatively depleted of hydrocortisone, and following the first infusion given when his tissues presumably contained normal amounts of this steroid. There was a difference between the manner in which creatinine, fluid, and nitrogen were handled in the first eight hours after infusion in these two studies, the explanations for which are not clear. The failure of potassium retention to persist during the last sixteen hours of each 48-hour period, at a time when sodium diuresis was occurring and hydrocortisone deficiency was probably present, is of interest and suggests a relationship with the nitrogen loss which was occurring at this time. The consistency of the nitrogen loss in the last twenty-four hours of each study was impressive.

The relationship of the observed metabolic changes to hydrocortisone levels in the blood is of special interest. Whereas effects upon potassium and creatinine excretion were completed by the time blood levels of the free steroid had returned to normal, sodium retention and nitrogen loss persisted well beyond this time, and in the patient with adrenal insufficiency even beyond the time when blood steroid levels had become subnormal. This observation is difficult to explain in any way other than a persistent steroid effect in the tissues in spite of a normal or subnormal level of free steroid in the blood.

It should be noted in this regard that with all three infusions the peak plasma levels of hydrocortisone, observed at the end of the infusions, ranged from 102 μg. to 165 μg. per 100 ml. This indicates that at this time only approximately 4 per cent of the administered steroid remained in the circulation in the free form. Furthermore, only approximately 4 per cent of the administered dose was excreted in the urine as free steroid and 20 per cent as conjugated 17,21-dihydroxy-20-ketones. These figures are compatible with the findings of Hellman and his associates (25), of Peterson and Wyngaarden (26), and of Brown and his associates (27). The appearance of conjugated steroids in the urine in relatively large quantities during the first fifteen minutes of the infusion in this study (22) indicates

the rapidity with which conjugation of this steroid occurs. The reports of others (26–30) suggest that the liver plays a role in this process.

With this evidence that conjugation of administered hydrocortisone occurs rapidly, and since only a small amount of the steroid remains in the free state in the circulation, it seems possible that a portion could have been producing metabolic effects in the tissues after the blood level had returned to normal or even subnormal values.

There are at least two clinical implications of the findings observed in this study. First, since elevated blood levels of hydrocortisone had returned to normal by the eighth hour after infusion, and since the time-interval is similar for the return to normal of blood levels of 17-hydroxy-corticosteroids after administration of hydrocortisone orally (31), a schedule of administration at maximum intervals of eight hours appears advisable when persistent elevated blood levels are desired. This situation might occur in the treatment of the adrenogenital syndrome or of metastatic mammary or prostatic carcinoma, when persistent inhibition of corticotropin is desired. Secondly, the timing of the metabolic changes observed affords an indication of the length of the time period after the last dose of hydrocortisone before one may expect withdrawal effects in a person with adrenal insufficiency, either due to disease of the adrenals, operative removal of the adrenals, or therapeutic suppression by long continued administration of cortisone, hydrocortisone or related steroids.

SUMMARY

The metabolic effects of a single intravenous infusion of 100 mg. of hydrocortisone over thirty minutes to a subject with normal adrenals and to one with adrenal insufficiency have been studied. Changes in urinary excretion of sodium, potassium, nitrogen, creatinine, fluid, and free and conjugated 17,21-dihydroxy-20-ketones were followed for forty-eight hours. These were correlated with plasma hydrocortisone levels. The subject with adrenal insufficiency received two infusions—one three hours after he had been receiving maintenance hydrocortisone and his tissues presumably contained normal amounts of this steroid, and the other forty-eight hours after the last dose of hydrocortisone, at a time when his tissues were presumably depleted of this steroid.

Plasma hydrocortisone levels reached peaks of 102 μg. to 165 μg. per 100 ml. at the completion of each of the three infusions. There was a return to the normal levels by the eighth hour after infusion in the normal subject and in the patient with adrenal insufficiency after he had been receiving maintenance oral hydrocortisone therapy, and a return to almost the normal level by this time in the latter patient after the second infusion administered forty-eight hours after his last dose of hydrocortisone. Subse-

quently the levels fell to subnormal values after both infusions received by the patient with adrenal insufficiency.

The effects upon urinary sodium and nitrogen excretion were relatively prolonged, lasting well beyond the time that levels of free plasma hydrocortisone had returned to normal, whereas effects upon urinary potassium, creatinine and fluid were shorter, being completed by the eighth hour after infusion. It is therefore evident that some of the actions of the steroid persisted in the tissues after blood levels had returned to normal or subnormal values. Furthermore, the changes in excretion of sodium, potassium, nitrogen and creatinine bore no constant relationship to one another.

Although there were some differences in the degree and duration of changes in urinary excretion of sodium, potassium, creatinine, fluid and nitrogen, the general patterns of the changes observed after the three infusions were similar.

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INVASIVE FIBROUS THYROIDITIS (RIEDEL'S STRUMA)*

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INVASIVE fibrous thyroiditis as herein defined is a rare clinicopathologic entity. The exact incidence cannot be ascertained readily from the literature, largely because many authors have not defined accurately the type of "thyroiditis" being reported. Thus, critical appraisal of all purported cases is necessary. Prior to 1932, it was widely held that Riedel's struma was an end stage of Hashimoto's disease. Graham (1), in a careful review of the literature up to 1929, emphasized the striking differences between the two diseases and considered only 41 cases of Riedel's struma (2) reported up to that time as authentic.

Since Graham's review, surprisingly few additional cases have been reported (3). A considerable number of reports on Riedel's struma deal in part or entirely with granulomatous (subacute) thyroiditis (4–11). The number of cases in which a strict definition of invasive fibrous thyroiditis is adhered to is extremely small. Schilling (12) described 1 authentic case with necropsy findings and 1 additional case obtained from 3,750 admissions for thyroid disease in a study of 8,000 necropsies. Lindsay and associates (13) reported 2 cases of invasive fibrous thyroiditis occurring in a total of 6,571 thyroidectomies over a 30-year period. Perhaps the largest well-documented single series is that of Joll (14), in 1939, who described 5 previously unreported cases in a series of 5,650 thyroidectomies.

It is equally difficult to gain an accurate picture of the clinical findings in Riedel's struma from the literature, since most series contain a high proportion of cases of granulomatous thyroiditis. Most authors stress that Riedel's struma is characterized by features of severe tracheal or esophageal obstruction, which occurs early and dominates the clinical picture

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(7, 12, 14, 15). In general, treatment has been directed toward removal of a localized fixed mass when the disease is unilateral and wedge resection of the isthmus to decompress the trachea when diffuse involvement is present. Crile (16) advocated splitting the involved lobe, followed by enucleation of a central adenoma.

A review of our material was considered desirable in order 1) to determine the incidence of Riedel's struma in a large series of cases of thyroiditis, 2) to obtain follow-up data on these patients so as to learn the natural history of this rare disease, and 3) to study the clinicopathologic features of this disease that distinguish it from granulomatous and other types of thyroiditis.

MATERIALS AND METHODS

The records in all cases of thyroiditis at the Mayo Clinic in which surgically removed thyroid tissue was available over a period of thirty-six years (1920 through 1955) were studied. All gross specimens of thyroid were available for review. Cases of exophthalmic goiter with associated lymphocytic infiltration or "thyroiditis" were excluded. Cases recorded as adenomatous goiter were reviewed only when the routine pathologic report mentioned a significant degree of associated thyroiditis, either fibrous or lymphocytic. The latter cases and all cases originally diagnosed as "thyroiditis" of any type were restudied.

All gross specimens were examined. Both lobes of the thyroid were cut at various levels and carefully inspected. Four additional blocks, on the average, were obtained in each case for histologic study. This careful gross inspection of each surgical specimen, noting especially any increased fibrosis or scar tissue in the thyroid, either focal or diffuse, was an invaluable aid in detecting possible cases of Riedel's struma. The gross examination of the specimen, combined with adequate histologic sampling, was sufficient to exclude from the study all typical cases of struma lymphomatosa and the more numerous examples of nonspecific chronic lymphocytic thyroiditis. There remained all cases in which fibrosis was a prominent feature in the gross and microscopic examination. In the latter cases, the gross specimen, numerous histologic sections, the clinical history and the notes recorded during surgical dictation were reviewed. Finally, the cases were classified into the following groups: 1) invasive fibrous thyroiditis (Riedel's struma); 2) granulomatous thyroiditis; 3) fibrous variant of struma lymphomatosa, and 4) focal scarring.

DEFINITION OF INVASIVE FIBROUS THYROIDITIS (RIEDEL'S STRUMA)

It is admitted that granulomatous thyroiditis and Riedel's struma possess a number of features in common, including hardness or firmness of the gland, but the differences are sufficiently pronounced to allow the two entities to be separated clearly. Likewise, the fibrous type of struma lymphomatosa, although grossly fibrous and firm, bears little other resemblance to the disease under consideration. In order to have a lesion included as invasive fibrous thyroiditis (Riedel's struma) in our study, the following morphologic features were required:

1. The fibrotic process might involve the entire thyroid gland or be

localized to one lobe or portion thereof. Grossly, the affected zone was tough, fibrous and woody.

2. The inflammatory fibrotic process had extended beyond the capsule of the gland into the surrounding structures of the neck. This extension usually was described clearly in the surgeon's notes, but microscopic proof of invasion and replacement of adjacent strap muscles or other tissue was required in all cases.

3. Microscopically, the inflammatory fibrotic process had caused nearly complete destruction of the involved portion of the thyroid. The involved zone showed no lobulation or discernible thyroidal capsule. The manner of involvement and destruction of the thyroid appeared unique, in that it was typified by total absence of the giant-cell reaction seen in granulomatous thyroiditis. The contrast in this respect between the two diseases was particularly striking in the junctional zone between normal thyroid and the involved tissue.

Thus, the histologic findings in invasive fibrous thyroiditis, as used in this study, differ from those in granulomatous thyroiditis in two main respects: 1) invasive fibrous thyroiditis usually causes more nearly complete destruction of the involved thyroid tissue, with absence of giant-cell or granulomatous reaction, and 2) the inflammatory process in invasive fibrous thyroiditis extends beyond the thyroid into adjacent structures, frequently to a pronounced degree. In contrast to invasive fibrous thyroiditis, the fibrous type of struma lymphomatosa shows absence of extrathyroidal extension, preservation of some degree of lobulation in the gland, an intact capsule and the persistence of islands of oxyphilic epithelium throughout the gland. Lesions belonging to the fourth group, namely focal scars of unexplained origin, were observed occasionally in gross examination of the tissues.

RESULTS

A detailed study of the cases of invasive fibrous thyroiditis (Riedel's struma) only are included in this paper. A study of the clinical and morphologic features of granulomatous thyroiditis will be reported later.

Incidence. Twenty cases of invasive fibrous thyroiditis (Riedel's struma) in our material fulfilled the aforementioned requirements. All the remaining cases in which fibrosis was prominent in the gross specimen could be classified as granulomatous thyroiditis, either recent or ancient, or a fibrotic type of struma lymphomatosa. These 20 cases occurred in a 36-year period during which approximately 42,000 thyroidectomies were performed; thus, they represent an incidence of about 1 in 2,000 thyroidectomies (0.05 per cent). The remaining cases of thyroiditis during this period totaled approximately 760, of which about 100 were granulomatous in nature.

Age and sex. These 20 cases of Riedel's struma included 16 women and 4 men. The ages ranged from 30 to 67 years, with an average of 49.5.

Duration of goiter. The duration of the goiter varied from two months to about five years (Table 1). The duration of symptoms in 11 cases was two to six months, with an average of 3.4 months.

Clinical and laboratory findings. A universal presenting complaint of this series of patients with invasive fibrous thyroiditis was a lump in the neck.

TABLE 1. CLINICAL DATA IN RIEDEL'S STRUMA: 20 CASES

Case	Date of operation	Patient		Duration of goiter	Pressure symptoms	Thyroid status on admission	BMR (%)	Sed'n rate, mm. first hr. (Westergren)	Preoperative diagnosis
		Age (yr.)	Sex						
1	Sept. 17, 1955	39	F	3½ mo.	Dysphagia	Myxedematous	−25	138	Riedel's struma; exclude carcinoma
2	May 30, 1949	53	F	11 mo.	0	Euthyroid	+8	31	Riedel's struma or carcinoma
3	Jan. 9, 1945	38	M	4 to 5 yr.; recent growth 9 mo.	0*	Myxedematous	−5	119	Riedel's struma or carcinoma
4	Sept. 19, 1941	30	F	4½ mo.	0 (Heavy sensation in neck)	Myxedematous (?); getting thyroid	−11	38	Riedel's struma; possible carcinoma
5	Feb. 2, 1933	43	F	6 mo.	0	Myxedematous	−17	—	Riedel's struma; consider carcinoma
6	Dec. 26, 1923	52	F	5 yr.; recent growth 1 yr.	0	Myxedematous	−5	—	Carcinoma versus thyroiditis
7	July 11, 1929	45	F	6 mo.	0	Euthyroid	−28 and −14	—	Probable carcinoma; consider thyroiditis
8	Aug. 28, 1943	44	F	2 mo.	Dysphagia	Euthyroid	−16	14	Carcinoma; possible thyroiditis
9	June 21, 1944	53	F	2 yr.	Dysphagia, feeling of pressure	Euthyroid	+4	28	Carcinoma (?)
10	July 6, 1922	52	F	5 yr.; recent growth 3 mo.	0	Euthyroid	+4	—	Nodular goiter; exclude carcinoma
11	May 28, 1926	67	M	2 mo.	0	Euthyroid	−7	—	Thyroiditis; exclude carcinoma
12	May 22, 1946	55	F	2 mo.	0	Euthyroid	−4	—	Riedel's struma
13	Dec. 6, 1920	55	F	3 mo.	0*	Euthyroid	—	—	Carcinoma or thyroiditis
14	June 20, 1935	44	F	1 yr.	0	Euthyroid	+12	—	Carcinoma or thyroiditis
15	Aug. 22, 1924	62	F	4 mo.	Slight dysphagia, sense of pressure	Euthyroid	+7	—	Probable carcinoma; thyroiditis(?)
16	Apr. 26, 1939	58	F	3 yr.; recent growth 2 yr.	0	Euthyroid	+6	66	Thyroiditis; possible carcinoma
17	June 17, 1939	38	M	14 mo.	0	Euthyroid	−7	24	Riedel's struma; carcinoma (?)
18	Dec. 2, 1952	65	F	2½ mo.	0	Euthyroid	+7	51	Carcinoma or thyroiditis
19	Dec. 7, 1951	51	F	2 mo.	0*	Euthyroid	−7	37	Probable carcinoma; thyroiditis (?)
20	Mar. 9, 1953	45	M	2 yr. (gradual growth)	0	Euthyroid	−4	6	Carcinoma (?)

* Slight thyroidal tenderness.

Pain in the region of the thyroid was absent in all cases, as was referred pain to the region of the jaw or ear. The lump was described as being tender or slightly tender to palpation in 3 cases. Symptoms of obstruction were not common; 4 patients complained of dysphagia, usually of slight degree, and only 1 of dyspnea, which was not regarded as obstructive in type. Two of the patients who had dysphagia also complained of a "heavy sensation" or a "sense of pressure" in the neck. An additional patient complained of soreness and a choking sensation. Four patients were definitely myxedematous on admission; a fifth probably had myxedema but was taking thyroid. The remainder were euthyroid. The goiter was considered bilateral in 11 cases and unilateral in 8, the status not being stated in 1. The goiter in all instances was described as extremely hard. Fixation in the neck was noted in 10 patients. Carcinoma and fibrous thyroiditis were given approximately equal weight in the preoperative diagnoses (Table 1).

Basal metabolic rates in these patients ranged from −28 to +12 per cent. Erythrocytic sedimentation rates were determined on 11 patients and varied from 6 to 138 mm. during the first hour (Westergren method).

Surgical and pathologic findings. Although the basic pathologic changes were histologically identical in all cases, the extent of thyroidal involvement and the degree to which extrathyroidal structures were infiltrated by the inflammatory process varied considerably from case to case. Since the entire thyroid gland was seldom available for study, a combination of clinical features, the description of the gland at operation and a careful histologic study of the resected portion of the gland provided a reasonably accurate basis for determining the extent of involvement and the presence or absence of a central adenoma. It is noteworthy that the surgically resected tissue in every case included striated muscle that was involved in the process, and in no case could a line of demarcation or a capsule be demonstrated between what was originally thyroid tissue and contiguous muscle.

In 6 cases (Cases 1–6, Tables 1 and 2), the disease apparently involved the entire thyroid gland at the time of operation. Fixation in the neck was noted in 3 of these. Infiltration of adjacent muscles was specifically described by the surgeon in 3 cases, and the process in 1 additional case was said to merge with the trachea. In 1 case, the status of adjacent structures was not mentioned but the condition was considered carcinoma and described as clearly inoperable. Surgical treatment in 5 of these 6 cases consisted of wedge resection of the thickened isthmus to free the trachea. This procedure usually included resection of some adjacent tissue from the inner border of each lobe. In the sixth case, most of the right lobe was resected and biopsy was performed on the left.

Evidence in favor of complete involvement and destruction of all thyroid tissue in these 6 cases was provided by the clinical and surgical find-

ings, combined with the presence of preoperative myxedema in 5 cases and postoperative myxedema in the remaining case after wedge resection of the isthmus. Extrathyroidal extension was present in all cases. Pathologic findings were identical in all 6 cases and Case 1 is outlined as typical.

The thyroid gland in Case 1 was described by the surgeon as fixed in the neck and extremely hard. The fibrous process was not confined by the capsule of the gland but blended imperceptibly with the surrounding muscles. By the process of slicing away segments of isthmus and adjacent thyroid, the trachea was freed and the firm mass of dense fibrous tissue that had replaced the normal thyroid was divided into halves at the trachea (Fig. 1*a*). Resected tissue included the isthmus, adjacent inner aspect of the thyroid lobe and fused muscle; it aggregated 25 Gm. Grossly, these slices were white, hard and avascular, bearing no resemblance to normal thyroid tissue (Fig. 1*b*). Involvement and destruction of the strap muscles were noted. There was no capsule and it was impossible to ascertain the actual borders of the previously existing thyroid.

Histologic study of what previously was thyroidal parenchyma revealed complete destruction and replacement by inflammatory fibrous tissue. The fibrous tissue in much of the involved region was hyalinized and relatively acellular (Fig. 2*a*). This was interspersed with numerous zones containing considerable infiltration by inflammatory cells, mainly polymorphonuclear leukocytes (Fig. 2*b*). Some plasma cells were present, as well as an occasional collection of lymphocytes (Fig. 2*c*). Virtually no evidence of previously existing thyroid tissue was found. Microscopic examination of a slice of tissue measuring 4.5 by 2 cm., representing a transection through the entire thickened thyroidal isthmus and adjacent lobe of thyroid, showed only one small collection of atrophic thyroidal acini which were engulfed by the inflammatory process (Fig. 2*d*).

Evidence of involvement of extrathyroidal structures was readily demonstrated in the resected tissue. The strap muscles were replaced by a process similar to that present in the thyroid (Fig. 3*a*). The fibers of striated muscle were separated by an intense inflammatory reaction, in the advancing edge of which the exudate consisted mainly of plasma cells, polymorphonuclear leukocytes and lymphocytes (Fig. 3*b* and *c*). This cellular exudate was gradually replaced by more acellular fibrous inflammatory tissue. The inflammatory fibrotic process surrounded blood vessels and nerves, as well as lymphoid tissue (Fig. 3*d* and *e*).

Adenomas or cystic cavities were not noted by the surgeon in any of these 6 cases and adenomatous tissue was not found in histologic sections of any of the resected tissue.

In 4 cases (Cases 7–10, Tables 1 and 2), although bilateral involvement was noted by both clinician and surgeon, the destruction of the thyroid gland was possibly incomplete. All 4 patients were euthyroid on admission. The surgical treatment in these 4 cases is outlined in Table 2. Postoperatively, 2 patients remained euthyroid and 1 became myxedematous; when subsequently heard from, the fourth patient was receiving thyroid but her status was otherwise unknown.

Pathologic examination of the surgically resected tissue revealed an inflammatory fibrotic process involving the thyroid and adjacent striated muscle, exactly similar to that already described for Case 1.

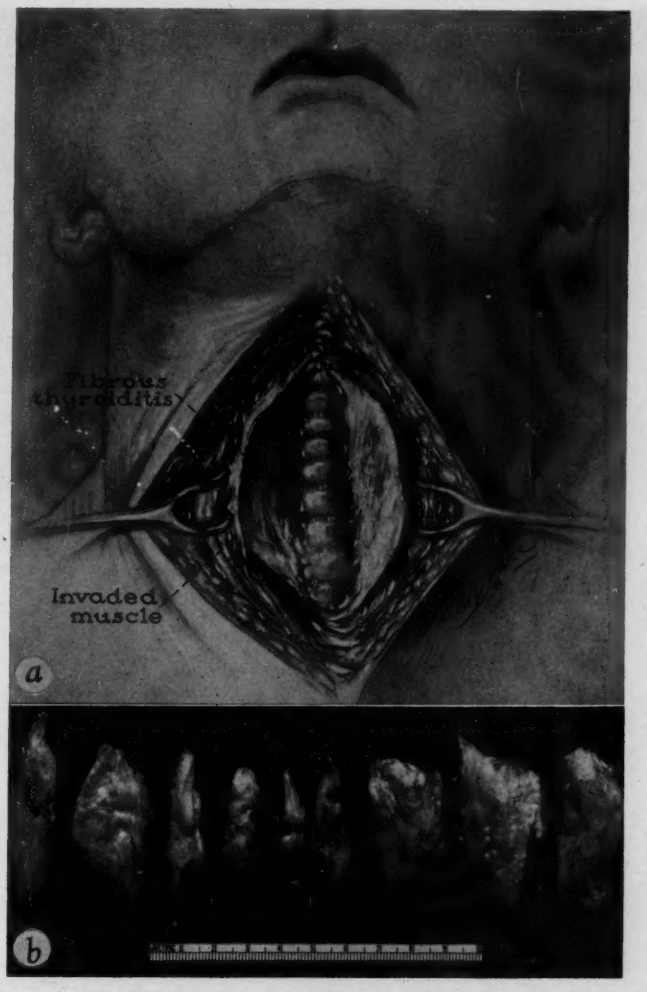

FIG. 1 (Case 1). *a*. Appearance of operative field at completion of operation. The usual collar incision was employed. *b*. Resected tissue. The white tough wooky slices represent replaced thyroidal parenchyma and adjacent involved strap muscle. There was no capsule.

The disease in 2 cases (Cases 11 and 12, Tables 1 and 2) was first unilateral, and the involved lobe had been resected. After an interval of one year to two years, this was followed by involvement of the second lobe. In Case 11, a hard fixed mass was present originally on the right side. The surgical procedure consisted of division of the isthmus and subtotal resec-

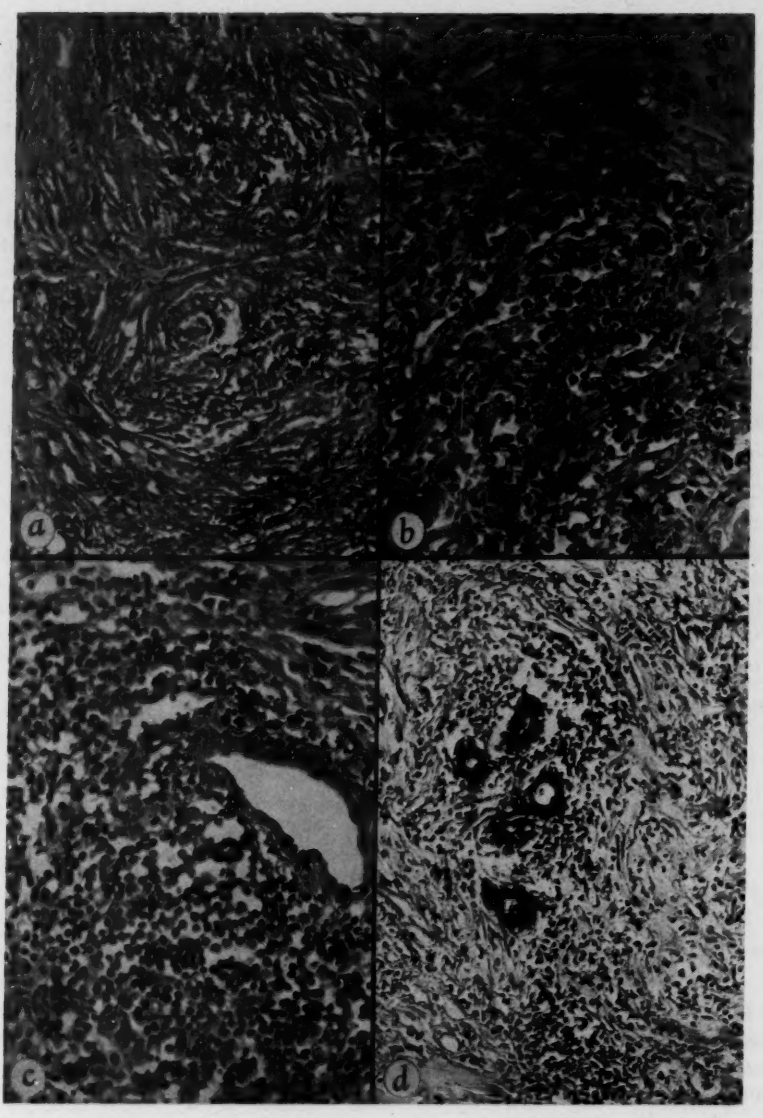

FIG. 2 (Case 1). Details of inflammatory process. *a.* Hyalinized, relatively acellular fibrous tissue (hematoxylin and eosin; ×120). *b.* Focus of polymorphonuclear infiltration (hematoxylin and eosin; ×250). *c.* Small zone of lymphocytic infiltration (hematoxylin and eosin; ×215). *d.* An island of persisting atrophic thyroid follicles (hematoxylin and eosin; ×140).

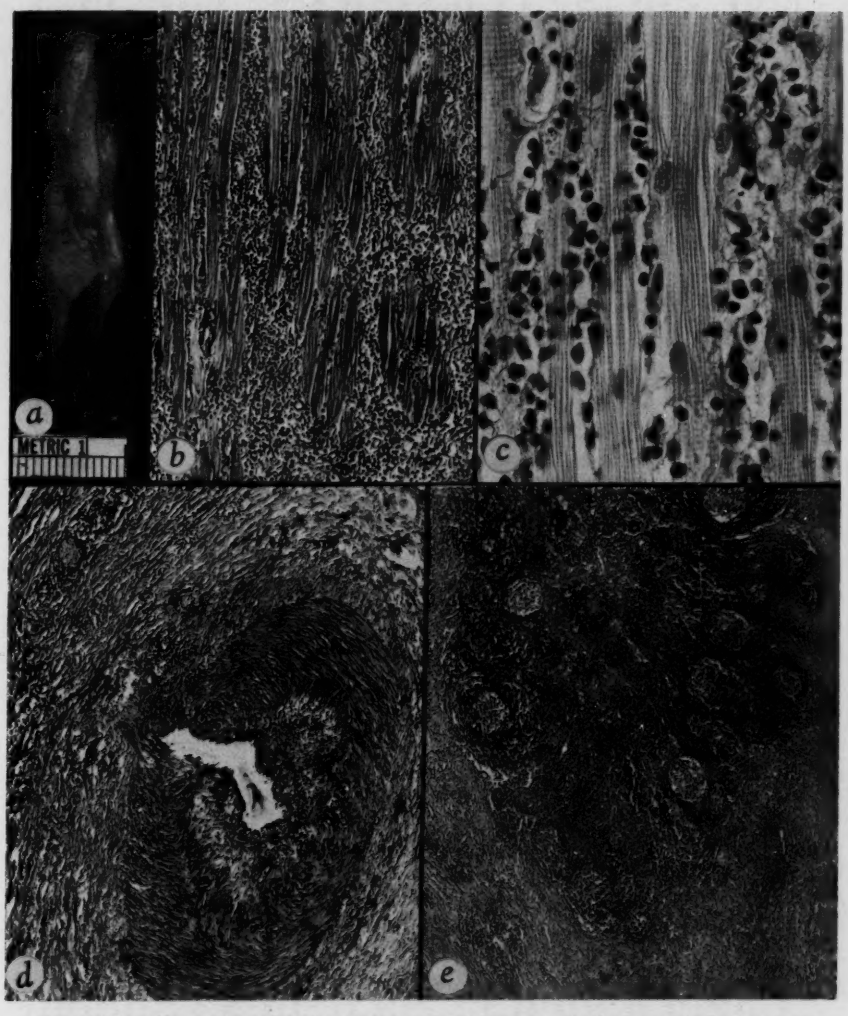

FIG. 3 (Case 1). *a.* Gross involvement and replacement of strap muscle. *b.* Separation of muscle fibers by cellular inflammatory infiltrate (hematoxylin and eosin; ×85). *c.* Higher magnification of same region, showing infiltration by polymorphonuclear leukocytes, lymphocytes and plasma cells (hematoxylin and eosin; ×400). *d* and *e.* Vessels, nerves and lymph nodes surrounded by the fibrous inflammatory process (hematoxylin and eosin; ×50 and ×35, respectively).

TABLE 2. SURGICAL AND FOLLOW-UP DATA IN RIEDEL'S STRUMA: 20 CASES

Case	Extent of lesion	Surgical description	Surgical treatment	Postoperative thyroid status	Course
1	Bilat.	Both lobes and isthmus involved by fibrosis, blending with surrounding muscles; impossible to remove	Wedge resection isthmus and adjacent portion both lobes	Myxedematous	Last seen Jan., 1956; on thyroid; mass in neck unchanged; no obstructive symptoms
2	Bilat.	Both lobes and isthmus 2½ times normal size; difficult to distinguish trachea from thyroid	Wedge resection isthmus and adjacent portion left lobe	Myxedematous	Last seen Aug., 1949; on thyroid; thyroid gland firm; no obstructive symptoms: last report Apr., 1956; good health; on thyroid; no obstructive symptoms
3	Bilat.	Very firm thyroiditis of entire gland and isthmus; extensive involvement of sternothyroid muscles	Trachea freed by wedge resection	Myxedematous	Last seen Nov., 1946; thyroid gland stony; on thyroid: last report Apr., 1956; still on thyroid; goiter same condition
4	Bilat.	Both lobes involved and very hard; infiltration of overlying muscles; impossible to remove	Wedge resection isthmus and inner border each lobe	Myxedematous, mild	Last seen July, 1949; both lobes palpable and firm; no dysphagia or dyspnea
5	Bilat.	Both lobes very large, hard, adherent to muscles; isthmus 5 cm. thick	Wedge resection isthmus to free trachea	Myxedematous	Last seen May, 1940; BMR-24; myxedema?; on thyroid; thyroid enlargement still present
6	Bilat.	Both lobes enlarged, hard, adherent to muscles; clearly ineradicable	Subtotal lobectomy right; biopsy left	Myxedematous	Last report 1934; on thyroid; no symptoms
7	Bilat.	Diffuse woody thyroiditis both lobes	Biopsy only	Probably myxedematous	Died 1938 after abdominal exploration elsewhere; pulmonary edema
8	Bilat.	Both lobes enlarged and extremely hard; more laterally; strap muscles adherent	Wedge resection isthmus and medial third each lobe	Euthyroid	Last seen Feb., 1951; BMR-14; thyroid not palpable; no symptoms
9	Bilat.	Very hard, especially on left; felt malignant	Several biopsies	Euthyroid	Last seen Sept., 1954; BMR +8; PBI 3.8 μg./100 ml.; lump in neck unchanged; no dyspnea or dysphagia
10	Bilat.	Both lobes involved, very firm; involving adjacent structures	Subtotal thyroidectomy	Myxedematous, left fixed cord	Last seen Feb., 1931; BMR-25; on thyroid; no symptoms; thyroid not palpable
11	Unilat., then bilat.	Right lobe enlarged, hard and adherent; left lobe normal	Subtotal resection right lobe	Euthyroid	Recurrence left lobe 2 yrs. after operation; biopsy=fibrous thyroiditis invading adjacent muscle; died 1935
12	Unilat., then bilat.	Left lobe enlarged 6 times normal, very hard; right lobe partially resected 1 year before	Left lobectomy	Euthyroid	Last report Mar., 1956; good health; no recurrence following 2nd operation; no obstructive symptoms
13	Unilat.	Right lobe 6–8 cm., densely hard, adherent to muscles and esophagus; clinically malignant; left lobe normal	Right total lobectomy	Euthyroid	Last report 1934; no symptoms relating to thyroid; no recurrence
14	Unilat.	Left lobe enlarged 5 times; involving adjacent muscles; right lobe normal	Subtotal left lobectomy	Euthyroid	Last seen 1936; no symptoms relating to thyroid: last report Apr., 1956; feeling well
15	Unilat.	Woody thyroiditis left lobe; muscles and internal jugular vein sacrificed in removal	Subtotal left lobectomy; isthmus removed	Euthyroid	Last report 1934; feeling well; no recurrence
16	Unilat.	Right lobe woody, 8–10 cm. in diameter; involvement of surrounding muscles; left lobe normal	Wedge resection isthmus; enucleation central adenoma right lobe	Euthyroid	Last seen Mar., 1952; no symptoms or recurrence
17	Unilat.	Explored 3 weeks before; now firm 10-cm. tumor right lobe; sternothyroid muscle in mass; cystic cavity right lobe	Wedge resection isthmus; anterior portion right lobe removed	Euthyroid	Last seen July, 1940; no complaints; BMR-9: last report Apr., 1956; good health; no further trouble from thyroid
18	Unilat.	Left lobe enlarged 4 times, stony; entire lobe fixed in neck; central degenerating adenoma	Subtotal left lobectomy	Euthyroid	Last report July, 1953; apparently well
19	Unilat.	Hard mass left lobe, involving ribbon muscles; right lobe normal	Subtotal left lobectomy; removal isthmus	Euthyroid	Last seen Jan., 1956; no symptoms or recurrence
20	Unilat.	Right lobe and isthmus hard and woody; beginning compression of trachea; jugular vein and carotid artery involved	Subtotal right lobectomy; removal isthmus; portion internal jugular vein sacrificed	Euthyroid	Last report Mar., 1956; good health; no symptoms; no goiter

tion of the involved right lobe. Two years later, a similar painless mass appeared on the left side of the neck. Surgical exploration revealed the left lobe to be enlarged six to seven times. Biopsy disclosed typical findings of Riedel's struma, with infiltration of adjacent strap muscle.

In Case 12, the right lobe of the thyroid had been removed elsewhere one year prior to admission. Histologic study of that tissue revealed Riedel's struma, with invasion of muscle. When the patient came to the clinic, a hard mass of two months' duration was present in the left lobe of the thyroid. This was surgically resected. Pathologic examination revealed fibrous thyroiditis involving approximately four fifths of the lobe (Fig. 4*a*). The fibrotic process blended imperceptibly into adjacent strap muscle. Adenomas were not found in the resected lobe.

Three cases (Cases 13–15, Tables 1 and 2) showed extensive or apparently complete involvement of one lobe of the thyroid gland. In Case 13, the surgically resected right lobe weighed 25 Gm. Pathologic examination revealed that approximately four fifths of the lobe was involved by fibrous thyroiditis (Fig. 4*b*). Adjacent muscle, fat, nerves and parathyroid gland were engulfed and involved in the fibrotic process (Fig. 4*c*).

In Case 14, surgical exploration disclosed fibrous thyroiditis involving the left lobe and adjacent muscles, trachea and esophagus. It was necessary to sacrifice a portion of the internal jugular vein in an attempt at removal.

In Case 15, a stony mass was present on the left side. Resection of the isthmus and subtotal left lobectomy were performed. The surgical specimen weighed 13 Gm. and was similar to the lesions in the preceding 2 cases.

In the remaining 5 cases (Cases 16–20, Tables 1 and 2), the process was unilateral and an adenoma or a cystic cavity, presumably the result of a degenerating adenoma, was present in the involved lobe.

In Case 16, the right lobe was hard and fixed, and it was considered impossible to resect the lobe surgically. The trachea was freed by division of the isthmus. Incision into the right lobe disclosed a large adenoma, which was enucleated (Fig. 5*a*). A large hole remained in the right lobe; its walls did not collapse because of the fibrosing inflammatory process. The resected tissue weighed 55 Gm. and consisted of a fetal adenoma surrounded by tissue involved by fibrous thyroiditis. Involvement of muscle was present. Normal thyroidal tissue was not found.

In Case 17, exploration of the neck had been carried out elsewhere three weeks prior to admission and the lesion had been considered inoperable. Surgical exploration at the clinic disclosed an extremely hard mass measuring 8 to 10 cm. in diameter on the right. The overlying sternothyroid muscle was incorporated in this mass. Incision into this lesion exposed a cystic cavity approximately 1.5 cm. from the surface. This cavity contained

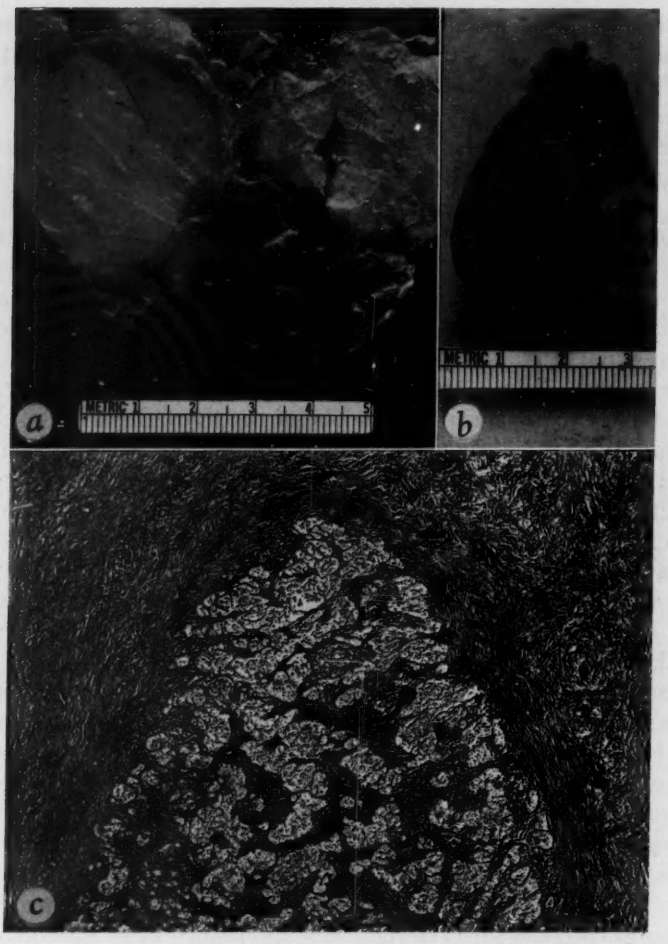

FIG. 4. *a*. Gross specimen (Case 12), showing replacement of approximately four fifths of lobe. *b*. Gross appearance (Case 13) of partially resected lobe. *c*. Adjacent parathyroid gland in another case surrounded and infiltrated by fibrotic process (hematoxylin and eosin; ×30).

about 75 ml. of slightly granular fluid. The entire lobe could not be removed, so the anterior portion was cut off, leaving a thickened rim surrounding the cystic cavity, the edges of which were sutured together. The trachea was exposed with great difficulty. The resected specimen weighed 13 Gm. It consisted entirely of a thick rim of tissue involved by fibrous

thyroiditis surrounding a cavity that apparently represented an ancient cystic adenoma (Fig. 5*b*). Microscopically, the process extended into striated muscle.

In Case 18, the surgically resected specimen weighed 10 Gm. Pathologic examination revealed a thick rim of tissue involved by fibrous thyroiditis surrounding a cystic cavity that apparently was the remains of a degenerat-

FIG. 5. *a*. Large adenoma (Case 16), enucleated from the involved lobe, together with the isthmus and involved striated muscle. *b*. Gross appearance (Case 17) of wall of cystic cavity in involved lobe. *c*. Gross appearance (Case 18) of resected specimen, showing wall of cystic cavity.

ing adenoma (Fig. 5*c*). Invasion of adjacent muscle was present.

In Case 19, subtotal removal of the left lobe was carried out, the resected portion weighing 26 Gm. Fibrous thyroiditis of a particularly dense type involved a portion of the lobe (Fig. 6*a*). A central colloid-containing zone had the appearance of an involutionary nodule. The overlying muscles were involved (Fig. 6*b*).

In Case 20, the resected specimen weighed 37 Gm., was extremely tough and woody and cut with great difficulty. A degenerating adenoma with a calcified rim 1.5 cm. in diameter was present, but not in the center of the fibrotic process (Fig. 7*a*). Histologically, the entire specimen consisted of acellular inflammatory fibrous tissue involving nerves, muscles and other structures; normal thyroidal tissue was not seen (Fig. 7*b* and *c*).

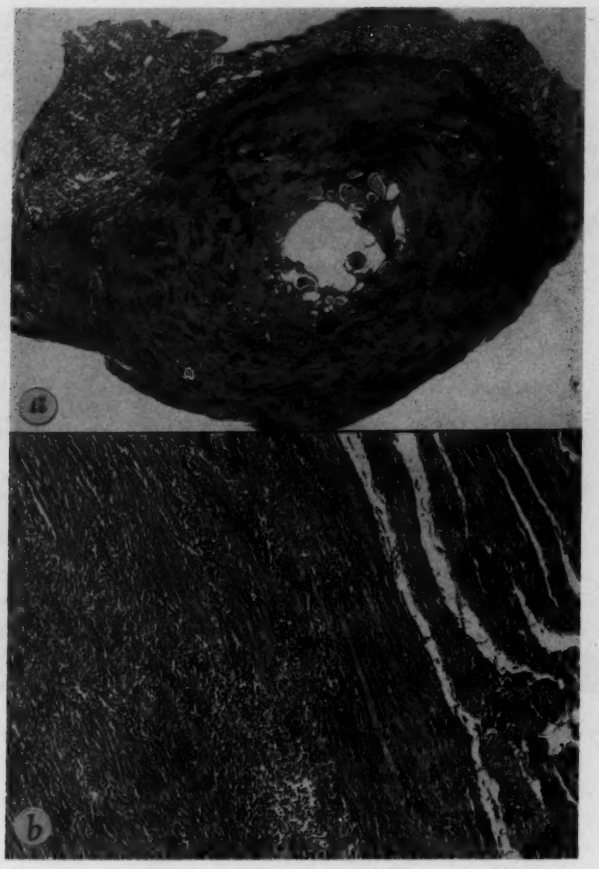

FIG. 6. (Case 19). *a*. Localized region of involvement of left lobe with central involutionary nodule (hematoxylin and eosin; ×2½). *b*. Section through adjacent muscle (hematoxylin and eosin; ×60).

SURGICAL TREATMENT AND COURSE

Surgical treatment in the presence of diffuse involvement consisted essentially of either wedge resection of the isthmus to free the trachea or biopsy only. Subtotal right lobectomy and biopsy of the left lobe were performed in 1 case, and bilateral subtotal thyroidectomy in 1 case. Subtotal or total lobectomy with or without removal of the isthmus was the surgical procedure employed when unilateral disease was present.

Follow-up data on these patients are listed in Table 2.

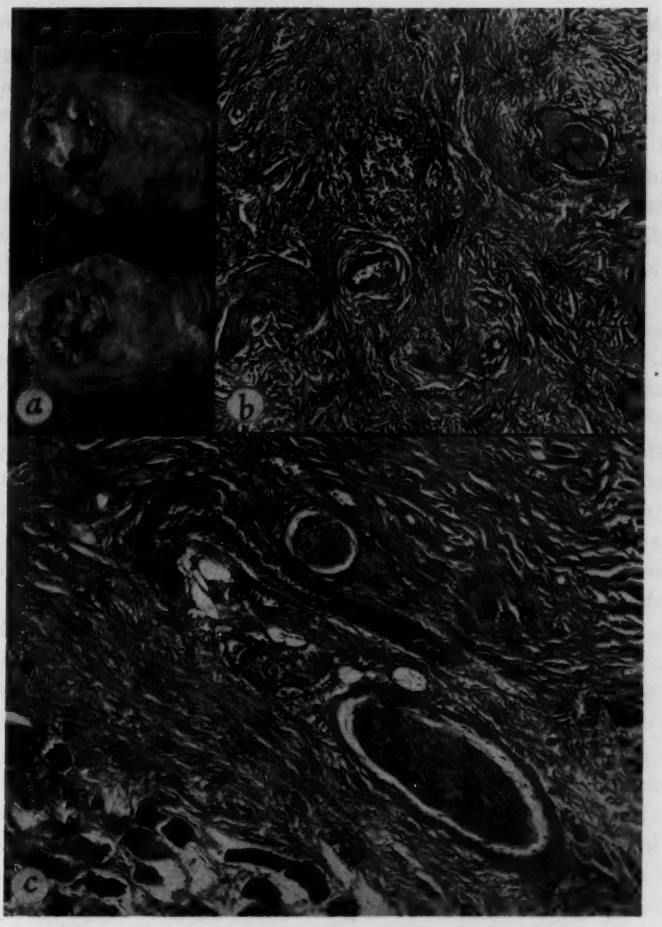

FIG. 7 (Case 20). *a*. Resected specimen, showing involvement of right lobe and isthmus. *b*. Section through thyroid, showing dense fibrosis (hematoxylin and eosin; ×85). *c*. Involvement of adjacent tissues (hematoxylin and eosin; ×70).

COMMENT

This study, based on material derived from approximately 42,000 resected thyroid glands, indicates that Riedel's struma, as herein defined, is an extremely rare disease. The incidence of approximately 1 per 2,000 thyroidectomies is about the same as that found in other published series in which a similar strict definition of the disease was adhered to. Struma

lymphomatosa and granulomatous thyroiditis are much more frequently observed, probably being ten to twenty times as common. Although rare, Riedel's struma appears to be a distinct entity and worthy of separation from the commoner forms of thyroiditis in which fibrosis is prominent, namely granulomatous thyroiditis and the fibrous variant of struma lymphomatosa.

Granulomatous thyroiditis (de Quervain's thyroiditis) produces a gland that is firm to hard; this lesion may be focal in type, involving a part or all of one lobe, with occasional bilateral involvement being observed. In this respect, it resembles Riedel's struma and frequently has been considered as a variant of this disease. However, some important differences exist. Clinically, granulomatous thyroiditis in its typical form is characterized by a painful tender thyroid gland; pain frequently is referred to the region of the ear or jaw. Systemic manifestations, such as malaise, weakness, loss of weight, fever and an increased sedimentation rate, are common. The duration of symptoms in surgically treated patients in our series was three weeks to two months. Riedel's struma, on the other hand, is characterized by a hard painless lump in the neck, sometimes associated with a sense of pressure or dysphagia. The main complaint is that of a goiter, the duration of which varies from a few months to several years.

Histologically, both Riedel's struma and granulomatous thyroiditis appear to be inflammatory in nature, and both destroy and replace thyroidal parenchyma. The destruction is typically much more nearly complete in Riedel's struma, in which few or no islands of persisting thyroidal parenchyma remain; such islands are commonly seen in the granulomatous type. A striking histologic difference between these two diseases is that Riedel's struma lacks a foreign-body giant-cell reaction, phagocytosis of colloid and tubercle formation (Fig. 8*a* and *b*). This holds true regardless of the duration of the disease as judged by the clinical history. Most important, the inflammatory process in Riedel's struma extends beyond the capsule into adjacent structures—a characteristic which may be considered the hallmark of this disease.

On surgical exploration, granulomatous thyroiditis also appears, like Riedel's struma, to be basically an inflammatory process, and adhesions may occur between the thyroid and adjacent structures. These vary from delicate, easily separated recent inflammatory adhesions to the unusual deposition of dense fibrous tissue that attaches the thyroid to muscles or other adjacent structures. However, a distinction is drawn between this adherence to adjacent structures and the extensive invasion that invariably occurs in Riedel's struma, making the latter condition basically an ineradicable one.

The explanation for the peculiar involvement and spread into adja-

cent cervical structures in Riedel's struma is not apparent. No other endocrine organ provides a parallel pathologic process. The only other inflammatory condition that might be considered somewhat similar, both grossly and microscopically, is the rarely encountered fibrous mediastinitis in which, on surgical exploration, an inflammatory fibrous process may be found to involve mediastinal structures extensively. The fibrotic process in Riedel's struma has been compared by some workers to the fibrosis that occurs in a desmoid tumor. However, the two processes are dissimilar in

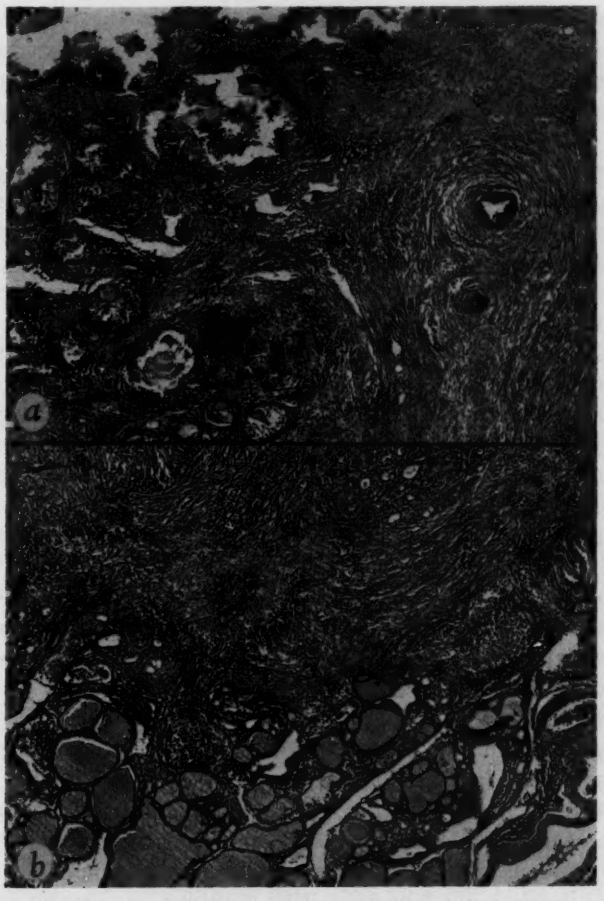

FIG. 8. *a.* Granulomatous thyroiditis showing pronounced giant-cell reaction to colloid at margin of thyroid tissue (hematoxylin and eosin; ×50). *b.* Histologic section through corresponding zone in Riedel's struma for comparison (hematoxylin and eosin; ×45).

that a desmoid tumor consists of a tumorlike proliferation of fibroblasts and lacks entirely the inflammatory component always present in Riedel's.

A surprising clinical finding was that most patients who had Riedel's struma complained on admission only of a painless unilateral or bilateral mass in the neck that might have been present from a few months to several years. The supposedly classic picture of symptoms of acute obstruction occurring early in the disease and dominating the clinical picture was not present in our cases.

The presence or absence of myxedema appeared to depend on the extent of involvement of thyroidal parenchyma. Most of the patients who had bilateral disease had myxedema on admission or had it after a minor resective procedure. By contrast, all patients in whom unilateral involvement was present were euthyroid on admission and remained so. When the isthmus only is removed, little change in the size or consistency of the goiter may be expected for a long period and subsequent symptoms of obstruction have not been observed. In patients who have unilateral involvement and in whom a considerable amount of the fibrous mass is removed surgically, recurrence of goiter is unlikely, although occasionally, as judged from 2 cases in this series, the disease may spread subsequently to the uninvolved lobe.

From the surgeon's viewpoint, patients who have Riedel's struma must be explored surgically because of a hard fixed mass involving one or both lobes of the thyroid. Although the white, woody, fibrous texture of the involved region may strongly suggest Riedel's struma on gross inspection, the diagnosis must be established by histologic examination of the involved tissue, preferably by immediate frozen section. An adequate amount of tissue should be removed for diagnosis. The biopsy procedure itself may be sufficiently extensive for the surgical treatment of the disease, which consists essentially of wedge resection of the isthmus and inner aspects of both lobes in the diffuse form, or partial removal of a localized mass when the disease is unilateral. Once the diagnosis is thus established, little reason exists for extending the surgical procedure farther in an attempt to remove all diseased tissue.

Follow-up information in our cases suggests that the process is self-limiting and that freeing the trachea provides a satisfactory guarantee against subsequent development of symptoms of obstruction, such as dysphagia or dyspnea. Although it is desirable cosmetically and as a means of securing adequate tissue for biopsy to resect a portion of the mass when the process involves one lobe only, little evidence was present in this series that the postoperative course of the disease is in any way dependent on removal of an adenoma during operation. Shelling out the lesion was performed in only 1 of the 5 patients in this series in whom an adenoma, in-

volutionary nodule or cystic cavity was found. In 1 patient, a small, partially calcified adenoma was present at one aspect of the resected tissue, and a central involutionary nodule was noted in 1 patient. The remaining 2 patients presented cystic cavities, presumably derived from old adenomas, in the center of the involved lobe. The subsequent course of the disease in these 5 patients did not differ in any respect from that in the remainder of the patients in the series.

SUMMARY AND CONCLUSIONS

Invasive fibrous thyroiditis (Riedel's struma) is an extremely rare clinicopathologic entity. A study has been made of 20 cases observed at the Mayo Clinic in a 36-year period, during which approximately 42,000 thyroidectomies were performed.

The presenting complaint of these patients was a hard lump in the neck, occasionally associated with a sense of pressure or dysphagia. Pathologically, the disease may be unilateral or bilateral, and extension into adjacent cervical structures is invariably present. A central adenoma, involutionary nodule or cystic cavity was not a prominent feature in this series.

The surgical treatment consists of wedge resection of the isthmus when the disease is bilateral or subtotal resection of an involved lobe as a biopsy procedure when the disease is unilateral. The prognosis after wedge resection of the isthmus or subtotal removal of a fibrotic mass involving one lobe appears to be excellent.

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METABOLIC STUDIES IN MYXEDEMA FOLLOWING ADMINISTRATION OF *l*-TRIIODOTHYRONINE: 1) DURATION OF NEGATIVE NITROGEN BALANCE; 2) EFFECT OF TESTOSTERONE PROPIONATE; 3) COMPARISON WITH NITROGEN BALANCE IN A HEALTHY VOLUNTEER*†

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THE administration of thyroid hormone to a patient with myxedema produces a marked negative nitrogen balance (1–4). The purpose of this paper is to present metabolic studies on the administration of *l*-triiodothyronine[1] to 2 patients with myxedema over a prolonged period, in an attempt to determine how long the negative nitrogen balance is maintained. The effect of testosterone propionate on the negative nitrogen balance induced by the *l*-triiodothyronine in the patient with myxedema, and the effect of *l*-triiodothyronine on nitrogen balance in a normal human volunteer, will also be discussed.

METHODS AND MATERIALS

The 2 patients with myxedema (H. J. and O. S.; case histories in Appendix) were studied while hospitalized on a metabolic unit, where they were ambulatory and allowed a relatively constant amount of activity daily. The healthy volunteer carried on his customary medical school activities but was fed on the metabolic unit. The subjects were maintained on a constant diet containing approximately 1 gram of protein per kilogram of body weight. All food was prepared and served from a metabolic kitchen. In the case of Patients H. J. and O. S., in whom multiple studies were carried out, the same diet was used for each study. During the periods that the patients were home between

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[1] Kindly furnished by Dr. Arthur Heming of Smith, Kline, and French Laboratories, Philadelphia, Pennsylvania.

studies, they were allowed an unrestricted diet but continued to take *l*-triiodothyronine in the same dosage as when in the hospital.

The nitrogen balance was determined by analysis of an aliquot of a 24-hour urine specimen, and an aliquot of a seven-day stool specimen, using the macro Kjeldahl technique. The diet was analyzed by the same technique at least once for each study.

Studies before and during *l*-triiodothyronine therapy were carried out in the following subjects: 1) H. J.: a 43-year-old white male with primary myxedema, 2) O. S.: a 31-year-

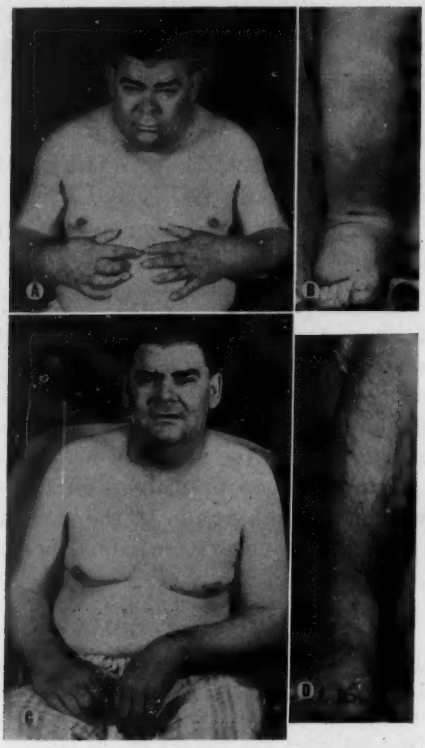

FIG. 1. *Patient H. J.* **A** and **B**, when myxedematous. **B** and **C**, after treatment with *l*-triiodothyronine.

old white female with primary myxedema, 3) S. A.: a healthy 26-year-old white male volunteer. L-triiodothyronine was administered as a single oral dose once daily. Testosterone propionate was given as a single intramuscular injection daily.

RESULTS

In *Patient H. J.* (Fig. 1) nitrogen balance was positive to the extent of about 2 grams daily prior to therapy (Fig. 2, Table 1). With *l*-triiodothyronine (T-3) therapy there was a definite shift in the nitrogen balance in the negative direction, which began within twenty-four hours after the

TABLE 1. DATA ON PATIENT H. J. (MYXEDEMATOUS MAN)

Dates	Inclusive days of study	Average daily nitrogen balance (Gm.)	Therapy
1955			
Aug. 7–12	1–5	+2.0	None
12–17	6–10	+1.4	None
17–22	11–15	+1.9	None
22–27	16–20	+2.3	None
27–Sept. 1	21–25	+1.9	None
Sept. 1–5	26–30	−0.3	*l*-triiodothyronine, 50 μg./day
5–10	31–35	−3.7	*l*-triiodothyronine, 75 μg./day
10–15	36–40	−5.2	*l*-triiodothyronine, 75 μg./day
15–20	41–45	−5.3	*l*-triiodothyronine, 75 μg./day
20–25	46–50	−6.0	*l*-triiodothyronine, 100 μg./day
25–27	51–52	−5.9	*l*-triiodothyronine, 100 μg./day
27–Oct. 2	53–57	−4.9	*l*-triiodothyronine, 100 μg./day Testosterone propionate, 25 mg./day
Oct. 2–7	58–62	−3.3	*l*-triiodothyronine, 100 μg./day Testosterone propionate, 25 mg./day
7–12	63–67	−2.4	*l*-triiodothyronine, 100 μg./day Testosterone propionate, 25 mg./day
12–16	68–71	−0.6	*l*-triiodothyronine, 100 μg./day Testosterone propionate, 25 mg./day
16–21	72–76	−1.5	*l*-triiodothyronine, 100 μg./day
21–26	77–81	−3.4	*l*-triiodothyronine, 100 μg./day
26–31	82–86	−4.5	*l*-triiodothyronine, 100 μg./day
31–Nov. 5	87–91	−2.1	*l*-triiodothyronine, 100 μg./day
Nov. 5–10	92–94	−1.7	*l*-triiodothyronine, 100 μg./day
	55 days at home; l-triiodothyronine continued		
1956			
Jan. 5–10	152–156	−1.7	*l*-triiodothyronine, 100 μg./day
10–15	157–161	−2.4	*l*-triiodothyronine, 100 μg./day
15–20	162–166	−1.2	*l*-triiodothyronine, 100 μg./day
	63 days at home; l-triiodothyronine continued		
Mar. 28–Apr. 2	230–234	−0.7	*l*-triiodothyronine, 100 μg./day
Apr. 2– 7	235–239	−1.3	*l*-triiodothyronine, 100 μg./day
7–12	240–244	−1.1	*l*-triiodothyronine, 100 μg./day
12–17	245–249	−0.2	*l*-triiodothyronine, 100 μg./day

first dose of triiodothyronine and became progressively greater during the first twenty-seven days, reaching a negative balance of 6 grams per day at this time. It will be seen (Fig. 2) that this was associated with a definite loss in weight, an increase in urinary output and a return of the BMR and serum cholesterol level toward normal. At this point, while keeping the *l*-

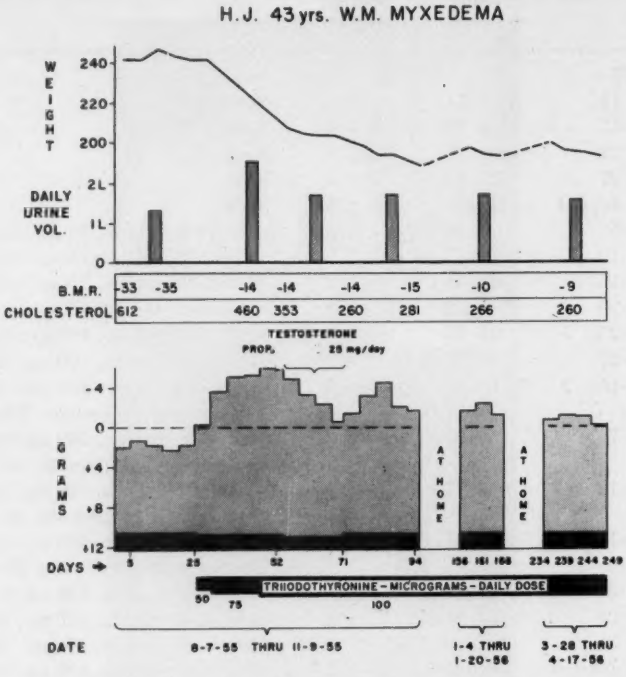

FIG. 2. Metabolic study on myxedematous Patient H. J., showing the effect of *l*-triiodothyronine and testosterone propionate on nitrogen balance.

triiodothyronine dosage constant at 100 micrograms per day orally, testosterone propionate, 25 milligrams daily, was administered intramuscularly for fourteen days. There was a prompt shift of the nitrogen balance in the positive direction; this became progressively greater and the balance became almost positive on the last day of testosterone propionate therapy. There was a decrease in the urinary volume but no significant weight loss or change in the BMR during this period. With the discontinuance of testosterone propionate there was a reversal of the nitrogen balance to the negative direction, but not to as great a degree as prior to androgen therapy. During the last ten days of the original study, the nitrogen balance became less negative with no concomitant change in therapy. At this point

(after ninety-four days of study) the patient was allowed to go home for fifty-five days, continuing to take *l*-triiodothyronine, 100 micrograms daily. He then returned to the hospital for fifteen days of additional study. It was found that nitrogen balance was still negative at about the level observed at the time of his original discharge. His weight had increased slightly during the interim at home. The patient was again allowed to go home for sixty-three days, continuing to take his medication. On resuming the study

TABLE 2. DATA ON PATIENT O. S. (MYXEDEMATOUS WOMAN) AND SUBJECT S. A. (NORMAL MAN)

Dates	Inclusive days of study	Average daily nitrogen balance (Gm.)	Therapy
		Patient O. S.	
1956			
Jan. 17–22	1–5	−0.6	None
22–27	6–10	−0.1	None
27–Feb. 1	11–15	−0.1	None
Feb. 1–6	16–20	−2.5	*l*-triiodothyronine, 100 μg./day
6–11	21–25	−2.7	*l*-triiodothyronine, 100 μg./day
11–13	26–27	−3.1	*l*-triiodothyronine, 100 μg./day
		81 days at home; l-triiodothyronine continued	
May 4–9	108–112	−2.0	*l*-triiodothyronine, 100 μg./day
9–14	113–117	−2.2	*l*-triiodothyronine, 100 μg./day
		Subject S. A.	
Nov. 1–6	1–5	+0.2	None
6–11	6–10	−0.7	None
11–16	11–15	−1.0	*l*-triiodothyronine, 105 μg./day
16–20	16–19	−1.5	*l*-triiodothyronine, 105 μg./day

after readmission to the hospital it was found that nitrogen balance was still negative, but to a lesser degree than before. During the last five days of his hospitalization (days 220–225 after T-3 therapy began) nitrogen metabolism was in equilibrium.

In *Patient O. S.* (female) nitrogen metabolism was in equilibrium prior to therapy (Table 2). In this patient also, there was a prompt shift of the nitrogen balance in the negative direction with the institution of *l*-triiodothyronine (T-3) therapy, 100 micrograms per day. The magnitude of the shift, however, was not as great as in Patient H. J. After twelve days of

therapy in the hospital, the patient was allowed to go home for eighty-one days continuing to take T-3, 100 micrograms per day. She was then rehospitalized and the study resumed. It was found that nitrogen balance was still negative after 101 days of T-3 therapy.

In *Subject S. A.*, a healthy volunteer, nitrogen metabolism was in equilibrium prior to *l*-triiodothyronine administration (Table 2). With the administration of T-3, 105 micrograms per day, there was an insignificant shift of the nitrogen balance in the negative direction.

DISCUSSION

Oral administration of *l*-triiodothyronine to a patient with myxedema produces a marked weight loss and a profoundly negative nitrogen balance, and this effect takes place within twenty-four hours (Fig. 2). Twenty-

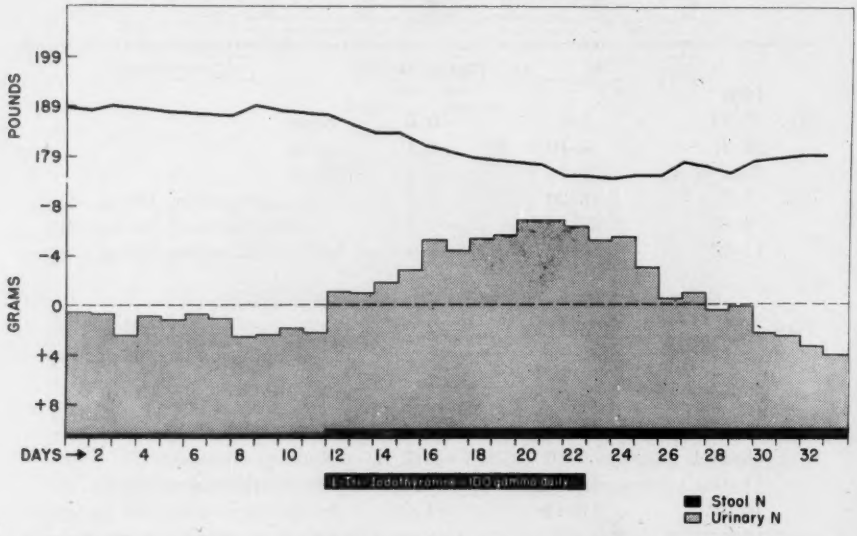

FIG. 3. Metabolic study on a patient with myxedema, showing the effect on nitrogen balance of the administration and withdrawal of *l*-triiodothyronine (5).

four hours after discontinuing the hormone the nitrogen balance returns toward the pretreatment level, as shown by data in another patient with myxedema from a previous study (5) (Fig. 3). A pronounced negative nitrogen balance was also obtained by Asper *et al.* (3) when *l*-triiodothyronine was given subcutaneously, and by Boothby *et al.* (1) using intravenous *l*-thyroxine. In our study, a comparable dose of oral *l*-triiodothyronine (100 micrograms daily) produced only a minimal negative nitrogen balance and no real weight loss (Fig. 4) in a healthy volunteer.

The duration of the negative nitrogen balance following the administration of thyroid hormone to a patient with myxedema has not been reported. Albright *et al.* (4) administered desiccated thyroid to a patient with myxedema and the patient still had a negative nitrogen balance at the end of forty-seven days. Our Patient O. S. still manifested negative nitrogen balance after 102 days of therapy with *l*-triiodothyronine. Patient H. J. was studied until nitrogen equilibrium was reached. As shown in

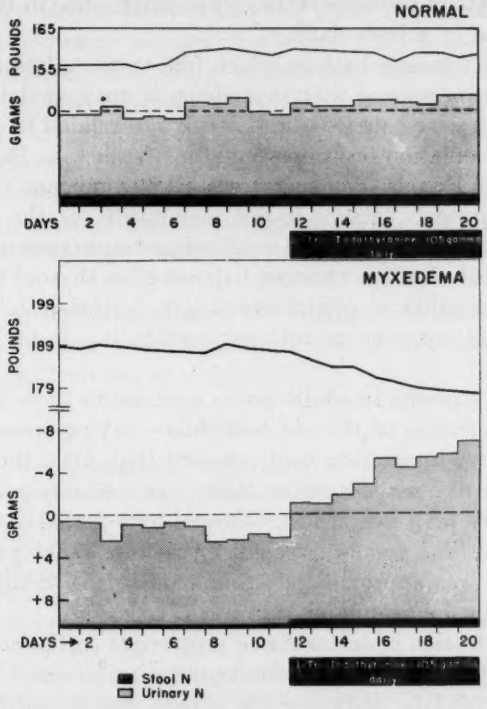

FIG. 4. A comparison of the effect of *l*-triiodothyronine on nitrogen balance in the patient represented in Figure 3 (myxedema) and in a healthy volunteer.

Figure 2, the negative nitrogen balance lasted between 200 and 220 days. It was our clinical impression that the patient was euthyroid at the end of six weeks. This was substantiated by a return of the BMR and the serum cholesterol concentration to normal levels. However, the negative nitrogen balance continued for at least 140 more days. It would appear that a myxedematous patient receiving replacement therapy maintains a state of negative nitrogen balance for a considerable period of time after he appears to be clinically euthyroid.

In Patient H. J., nitrogen balance was positive before the administra-

tion of *l*-triiodothyronine. This was a rather consistent finding in 6 of 8 patients that we have studied. Previous studies from our laboratory (5), using N^{15}-glycine, have shown that despite this positive nitrogen balance the rate of protein synthesis is markedly decreased as compared to that in healthy volunteers. This decreased rate of protein synthesis, like the negative nitrogen balance, also persists for a long period of time; but, as previously reported, it finally returns to normal after prolonged replacement therapy. The rate of protein synthesis was determined in Patient H. J. and will be reported at a later date.

The negative nitrogen balance which follows the administration of thyroid hormone to a patient with myxedema is not completely understood. The marked negative balance is apparently not related to excessive weight gain with accumulation of "myxedematous tissue," as Patient O. S. who weighed only 48 Kg. also demonstrated this phenomenon (Table 2). Previous positive nitrogen balance is not mandatory, as the patient studied by Albright *et al.* (4) had nitrogen equilibrium before treatment and showed the same marked negative nitrogen balance after thyroid therapy. We are continuing our studies in an attempt to gain further insight regarding the effect of thyroid hormone on nitrogen metabolism in myxedematous patients.

The foregoing results in adults are in contrast to those observed following the administration of thyroid to a child—having potential for growth. Wilkins in a very interesting study showed that, after the administration of thyroid to a $4\frac{1}{2}$-year-old cretin, there was a 15-day period of negative nitrogen balance with weight loss, followed by a period of marked positive nitrogen balance and a gain in weight for as long as the patient was being studied, which was approximately four months (6). Similar studies have been reported by Johnston and Maroney (7).

The administration of testosterone propionate to Patient H. J. resulted in a marked decrease in the previously negative nitrogen balance from an average loss of 6.0 Gm. daily to a loss of only approximately 0.6 Gm. daily (Fig. 2). There was little change in the degree of weight loss. Kinsell, Hertz and Reifenstein (8) have shown that in patients with thyrotoxicosis testosterone propionate will cause nitrogen retention. They stress the fact that there is a negative nitrogen balance in thyrotoxic patients, but in only 1 of their 3 patients was the nitrogen balance negative. In 1 patient there was a negative balance of 2.0 Gm. daily; a positive balance of 6 to 8 Gm. daily was produced with the testosterone propionate. It would be of interest to see the effect of this androgen in a thyrotoxic patient in whom there was a marked negative nitrogen balance. It appears that it causes nitrogen retention in thyrotoxicosis and also in myxedema when there is a negative nitrogen balance during thyroid therapy.

Testosterone propionate has been shown to produce an increase in basal metabolic rate in eunuchs (9, 10). There was no increase in basal metabolic rate in our patient with myxedema while receiving this drug (Fig. 2).

SUMMARY

1. The oral administration of 100 micrograms of *l*-triiodothyronine daily to a myxedematous man produced marked weight loss and a negative nitrogen balance which lasted between 200 and 220 days. Similar changes occurred in a myxedematous woman who weighed much less than the man, but they were neither so marked nor of such long duration.

2. The negative nitrogen balance persisted despite the fact that the patients appeared to be euthyroid clinically and other parameters of thyroid function indicated a return to normal levels.

3. In the man, the intramuscular administration of 25 milligrams daily of testosterone propionate caused nitrogen retention during the period of negative nitrogen balance. There was no change in the basal metabolic rate.

4. The daily oral administration of 100 micrograms of *l*-triiodothyronine for ten days to a healthy male volunteer produced insignificant changes in nitrogen balance and no loss of weight.

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APPENDIX

Patient H. J., a 43-year-old white man was admitted to the medical service of the University of Virginia Hospital on July 27, 1955. About nine years prior to admission he had begun to gain weight and noted the onset of fatigue, lethargy, and weakness. Over the following three to four years, cold intolerance, decreased sweating, swelling of his one leg, puffiness of the face, loss of hair, dryness of the skin, slowness of speech, hoarseness, poor memory and cerebration, decreased hearing, dyspnea on exertion without orthopnea, constipation, and arthralgia had developed. All of his symptoms had become progressively worse and during the prior year he had experienced impotence and loss of libido. There were no symptoms suggesting past thyroiditis. About eight years before admission he had been injured in a railroad accident, necessitating mid-thigh amputation of his left lower leg.

Physical examination revealed an obsese, lethargic, unkempt white male (Fig. 1) with a deep hoarse voice, immobile lips, and a very large tongue. His pulse was 85, blood pressure 150/110, and weight 240 pounds. The skin was cool, dry, thickened, and very scaly, with marked subcutaneous brawny edema. The skin of the face had a waxy pallid appearance. The scalp, axillary, and pubic hair was very sparse, dry and coarse. There were only a few dry short hairs scattered over the body and extremities. The face appeared massive because of subcutaneous periorbital and facial edema. The thyroid was not palpable. There was no venous engorgement. The lungs were clear. The heart was enlarged and the heart sounds were distant. An abdominal fluid wave was present. There was evidence of a mid-thigh amputation of the left lower extremity. The right foot and lower leg were massively swollen with brawny edema; the overlying skin was dry, scaly, greatly thickened, and pigmented. The deep tendon reflexes were hypoactive and sluggish.

The serum cholesterol level was 612 mg. per 100 cc. and the BMR was −33 per cent. An electrocardiogram revealed low voltage and T-wave abnormalities, consistent with myxedema. A chest roentgenogram revealed an enlarged heart with a cardiac-thoracic ratio of 63 per cent. Upon cardiac fluoroscopy, there was a huge heart of water-bottle configuration with diminished pulsations.

Patient O. S., a 31-year-old married white woman, was admitted to the University of Virginia Hospital on January 8, 1956. She gave a nine-year history of weakness, fatigue, constipation, cold intolerance, decreased sweating, skin dryness and pallor, periorbital fullness, diminution of scalp and body hair, slow speech, low-pitched voice, muscular stiffness, infertility, menstrual irregularity with menorrhagia, and anemia. The anemia had been unresponsive to various forms of therapy and all of the symptoms had been progressive to the time of admission.

The temperature was 97.4° F., pulse 64, blood pressure 90/70, and weight 100 pounds. Physical examination revealed apathy, slow movements and speech, low-pitched voice, dry cold skin, scanty dry scalp hair, thin brows laterally, absent axillary hair,

facial pallor, moderate periorbital edema, slight pretibial edema which pitted only with firm pressure, and sluggish tendon reflexes.

The initial hematocrit reading was 32 per cent, with normal indices: the reticulocyte counts ranged from 0.3 to 0.9 per cent. Examination of the bone marrow revealed mild hypoplasia; the serum iron and unsaturated iron binding, capacity were normal. The 24-hour thyroidal radioactive iodine uptake was 7.5 per cent, which did not rise after administration of thyroid-stimulating hormone. Her serum cholesterol level was 512 mg. per 100 cc., and the BMR ranged from −29 to −39 per cent prior to therapy. The electrocardiogram showed low voltage.

HORMONAL TREATMENT OF THYROID CANCER*

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THERE is considerable evidence that there may be two factors in the induction of malignant neoplasm (1). Certain stimuli (initiating factor) which alone are incapable of inciting tumor formation may so alter the cellular physiology and biologic potential that a sequential stimulus of a different sort (promoting factor) may be followed by neoplastic growth.

Experimentally, carcinoma of the thyroid fits closely into this scheme (2). A carcinogen alone (such as dibenzanthracine or 2-acetyl-aminofluorene) may induce very few thyroid tumors. If, however, this carcinogen is followed by a promoting factor (goitrogen) the incidence of tumor formation is strikingly high and much greater than when the goitrogen alone is administered. Most of these tumors remain dependent, but a few attain complete autonomy (3–5). Since in some instances these carcinomas appear to be under the influence of thyrotropic hormone, as long ago as 1947 Purves and Griesbach (6) suggested that, "It might be possible to influence the course of malignant thyroid disease in human beings by the therapeutic administration of desiccated thyroid."

The behavior of certain types of human thyroid cancer could be explained by the need of a promoting factor for neoplastic growth. In a thyroid gland with a genetic composition susceptible to tumor formation, introduction of a promoting factor might well be followed by neoplasia. It would account for the relatively higher incidence of papillary thyroid cancer in younger age groups subject to the physiologic stresses of puberty, adolescence and pregnancy, as well as the multiple foci of origin, and the increasing malignancy of the same tumor with advancing age.

These experimental and clinical considerations support the concept that cancer of the thyroid is not entirely an autonomous neoplasm. Certain cells during some period in their development may be dependent upon promoting factors, most likely thyrotropic hormone, for continued growth. If this be true, then alteration of the level of thyrotropic hormone may be re-

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flected in growth of the neoplasm. Since there exists a reciprocal relationship, elevation of the blood thyroid hormone concentration should be followed by a fall in the level of thyrotropic hormone (7). Evidence gleaned from suppression of the thyroidal I^{131} uptake, elevation of the serum protein-bound iodine concentration (8), and requirements for treatment of simple goiter and myxedema suggest that from 120 to 240 milligrams of desiccated thyroid daily will suppress the endogenous output of thyrotropic hormone by the anterior pituitary. In reviewing the accumulated experience of others (9–12), there is considerable evidence that in many instances regression of thyroid cancer followed the administration of thyroid hormone. Since frequently this was one of several forms of therapy employed, and in others the doses of thyroid employed were small and

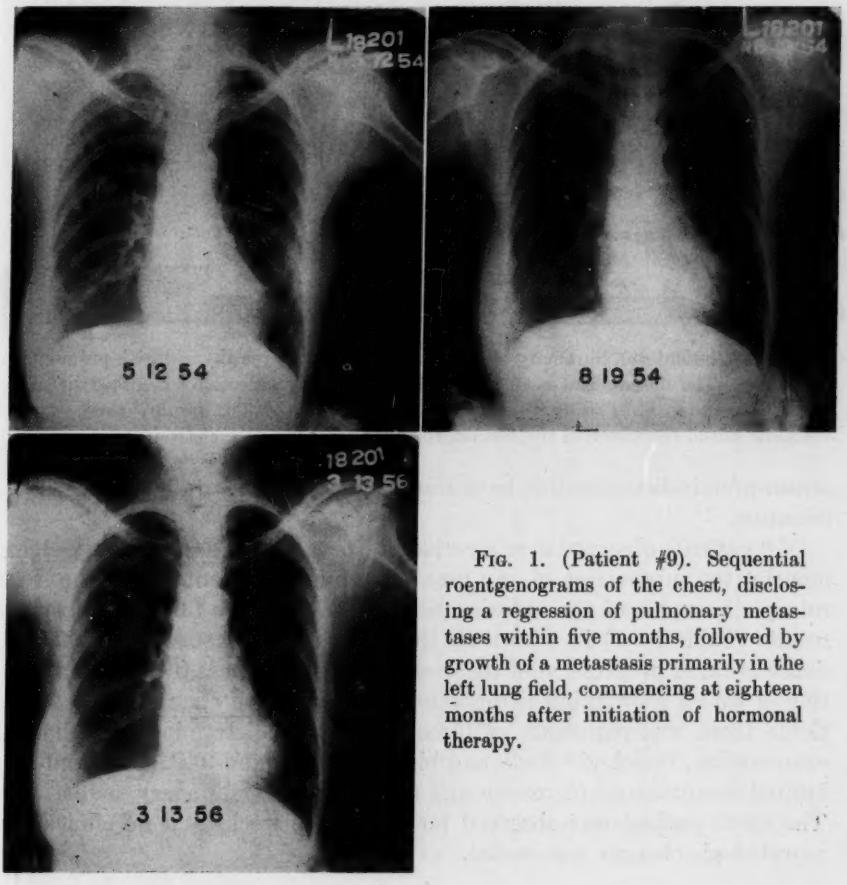

FIG. 1. (Patient #9). Sequential roentgenograms of the chest, disclosing a regression of pulmonary metastases within five months, followed by growth of a metastasis primarily in the left lung field, commencing at eighteen months after initiation of hormonal therapy.

designed only to maintain a euthyroid status, it has been difficult to evaluate solely the effect of thyroid hormone.

METHODS OF STUDY AND CLINICAL OBSERVATIONS

In order to determine the role of thyrotropic hormone in the growth of human thyroid cancer, patients with carcinoma of the thyroid were studied following the administration of sufficient desiccated thyroid to substitute for endogenous thyroid output; this varied from 120 to 300 mg. daily. In order to assure adequate substitution, an effort was made to depress the thyroidal I^{131} uptake to 5 per cent or below, and in some individuals the

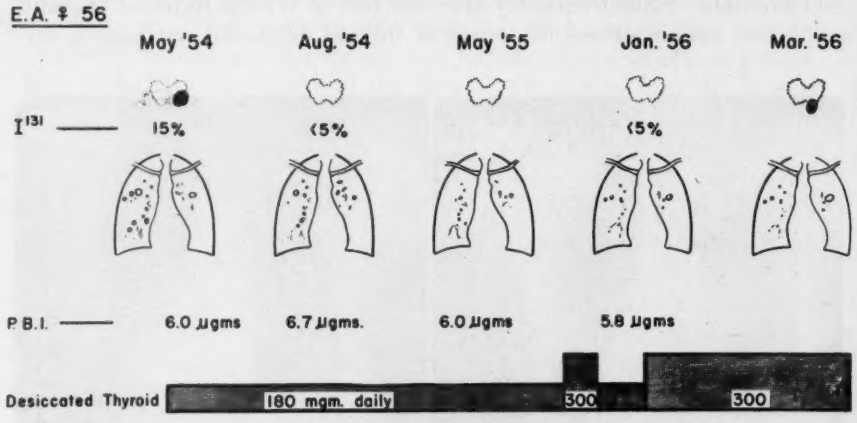

FIG. 2. (Patient #9). Summary of course of carcinoma of the thyroid with pulmonary metastases. The disease had been treated by partial thyroidectomy before desiccated thyroid was started. During twenty-eight months of thyroid therapy there was a dramatic initial regression of the disease, followed by recurrence at twenty-one months.

serum protein-bound iodine level was temporarily above the initial determination.

Of 9 patients observed over a period of from three weeks to twenty-eight months, the suppression of any promoting factor resident within the thyrotropic hormone of the anterior pituitary yielded the following varying results (Table 1): 1) In 3 patients there was no response and clinical evidence of rapid progression of the disease. 2) In 2 patients there was inhibition of tumor growth but little histologic evidence of change. 3) In 2 patients there was regression of tumor growth, as determined by clinical examination, radiologic study and biopsy (Figs. 1 and 2). One patient exhibited simultaneous regression and progression of pulmonary metastases. The ninth patient was observed for only three weeks and no clinical or morphologic changes were noted.

TABLE 1. SUMMARY OF HORMONAL TREATMENT OF 9 CASES OF THYROID CANCER

Patient number	Age & Sex	Type of carcinoma	Duration of disease	Extent of disease	Previous treatment	Daily dose of thyroid (mg.)	Duration of hormonal therapy	Other treatment	Result
1. D.F.	29 F	Papillary and alveolar	7 yrs.	Thyroid, cervical lymph nodes	None	180	3 wks.	Total thyroidectomy and radical neck dissection	No clinical or morphologic change
2. T.B.	36 M	Solid and alveolar adenocarcinoma	9 mos.	Thyroid, cervical lymph nodes, lung	None	180-300	9 mos.	X-ray to neck	Temporary regression of tumor in neck, then progression until death at 9 mos.
3. H.S.	70 F	Poorly differentiated adenocarcinoma	3 mos.	Thyroid, neck, cervical lymph nodes, lung	Total thyroidectomy, neck dissection	120-180	2 mos.	X-ray to neck	Rapid progression until death at 2 mos.
4. O.R.	48 M	Alveolar adenocarcinoma	7 yrs.	Left clavicle	Subtotal thyroidectomy, modified neck dissection	300	3 mos.	None	Continued growth of bony metastasis until excised. No morphologic change
5. L.R.	60 F	Alveolar, solid, and poorly differentiated adenocarcinoma	3 mos.	Thyroid, lung	Total thyroidectomy	120	6 wks.	None	Regression of metastases in right lung with rapid progression in left chest until death
6. C.S.	33 F	Papillary and alveolar adenocarcinoma	3 mos.	Thyroid, cervical lymph nodes	Total thyroidectomy, modified neck dissection	180	22 mos.	None	No evidence of progression by clinical examination
7. F.L.	65 F	Papillary and alveolar adenocarcinoma	1 yr.	Thyroid	Partial thyroidectomy for biopsy	120	22 mos.		No clinical evidence of progression. Questionable regression grossly and histologically at the time of re-exploration and biopsy
8. M.F.	31 F	Papillary and alveolar adenocarcinoma	1 yr.	Thyroid, lung	None	180-300	19 mos.	Total thyroidectomy after 1 yr. of hormonal therapy	Regression of pulmon. metast.; decrease in primary tumor. Improvement in clinical well being
9. E.A.	56 F	Alveolar adenocarcinoma	7 yrs.	Thyroid, neck, lung	Partial thyroidectomy	180-300	28 mos.	None	Dramatic initial regression of neck tumor and pulm. metast. Progression of primary tumor in neck and 2 of pulm. metast. at 21 mos.

CONCLUSIONS

Although all tumors in this series were not shown to be completely dependent upon thyrotropic hormone for continued growth, there seems to be supporting evidence that autonomy of growth in thyroid cancer is a quantitative phenomenon and is, in part at least, governed by its hormonal environment. In some neoplasms there may be cells which are still dependent on thyrotropic hormone for growth, and others which are more autonomous. Even though both elements may metastasize, they are still influenced in their growth by environmental factors. As with carcinoma of the breast and prostate, thyroid cancer is apparently capable of developing progressive autonomy of growth.

These observations would seem to have the following significance. If thyrotropic hormone can serve as a promoting factor in the development of thyroid cancer, the prolonged effect of excess thyrotropic hormone would seem hazardous. Most unwise would be the practice of repeated stimulation of thyroid cancer in an effort to induce greater uptake of radioactive iodine. Such stimulation might well enhance its autonomy. The practice of prolonged therapy with any of the antithyroid drugs, particularly in children in whom such therapy may be continued for several years (13), without the concomitant administration of exogenous thyroid should also be questioned. The role of this concept in the treatment of metastatic cancer needs further exploration. It would appear to have its greatest use in the treatment of patients who have already had the thyroidal carcinoma excised. Suppression of thyrotropic hormone in such a instance would be of value in 1) inhibiting any tendency of remaining normal thyroid tissue to undergo malignant change because of absence of promoting factor, and 2) inhibiting subsequent growth of any residual neoplasm for the same reason.

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THE RENAL CLEARANCE OF NEUTRAL 17-KETOSTEROIDS IN MAN*

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INTRODUCTION

APPROXIMATELY 15 to 26 per cent of the neutral 17-ketosteroids in normal human urine consists of dehydroepiandrosterone (1). The realization that, in human peripheral blood, by far the greatest portion of the neutral 17-ketosteroids occurs as dehydroepiandrosterone (2, 3) has aroused curiosity concerning the role of this compound in the intermediary metabolism of the adrenocortical steroids. The quantities of androsterone in human blood have been reported to be much smaller (3). Although it is possible that the dehydroepiandrosterone itself in human plasma is not derived primarily from the adrenal cortex (4), its preponderance in the blood has not been explained. These investigations were designed to study the renal clearance of the 17-ketosteroids in man and to determine whether renal factors might account for the high level of dehydroepiandrosterone in human blood.

METHODS

A normal human subject, aged 34 years, was used for most of these studies. Dehydro epiandrosterone or androsterone was administered separately in various doses by mouth; in one experiment both were administered together as the hemisuccinates by vein. Dehydroepiandrosterone acetate was administered orally in a dose of 2 Gm. dissolved in a small volume of hot ethanol, which was then rapidly diluted with a large volume of ice water immediately before use. Androsterone, free or as the hemisuccinate, was administered on several occasions by mouth in doses of 0.5 to 2.0 Gm. During each experiment large volumes of water were ingested in order to guarantee adequate volumes of urine, and several accurately timed periods of urine collection were established following administration of the steroid, at the midpoint of which specimens of blood were obtained in a heparinized syringe. The blood was centrifuged and the plasma was

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extracted by methods previously described (3). The extracts of both urine and plasma specimens were extracted continuously with ether at pH 1.0 for forty-eight hours. In certain instances hydrolysis was also carried out with beta-glucuronidase (beef liver) in acetate buffer (pH 4.5) and by dioxane, using the method of Cohen and Oneson (5). All residues were chromatographed on Florosil by the method of Gardner for 17-ketosteroids (6). The appropriate eluate was separated into alpha and beta fractions by digitonin precipitation (7). The alpha and beta fractions were then analyzed by various procedures: the Zimmermann reaction, according to the method of Wilson (8); the Pincus reaction (9); and the Allen method for dehydroepiandrosterone (10). Pooled fractions of blood, after the administration of either steroid by mouth, were examined by infra-red spectroscopy in order to guarantee that the residues were identical with the administered compound. The creatinine content of urine and plasma was determined by the method of Hare (11).

After the intravenous injection of steroids, duplicate extracts of plasma and urine were also separated by paper chromatography, using the iso-octane/70%-methanol system according to techniques described by the authors (12). The areas corresponding to androsterone and dehydroepiandrosterone, as determined by the positions of pure standards, were eluted from paper and the previous methods applied for their quantitative estimation. This was done in order to evaluate the correlation between paper chromatographic techniques and the methods otherwise employed. All measurements were carried out in accordance with the principles of Allen by skimming the peak (13). Readings were made with a Beckman DU spectrophotometer.

The hemisuccinates of androsterone and dehydroepiandrosterone were prepared by the method of Pincus and Pearlman (14) and were sterilized by filtration in physiologic saline solution through a Seitz filter prior to intravenous use. During the intravenous experiments alone, as detailed subsequently, both steroids were administered together.

In a single experiment following the oral administration of beta-cortolone (pregnane-3α,17α,20β,21-tetrol-11-one) the blood and urine were treated in a manner previously described (15), and the preparations were analyzed by periodate oxidation, utilizing the microformaldehydogenic method of the authors (16). In this single experiment, the Porter-Silber method (15) was applied for quantitation of 17,21-dihydroxy-20-keto compounds.

The renal clearances were calculated as follows:

$$\frac{\text{urine steroids } (\mu\text{g. excreted per min.})}{\text{plasma steroids } (\mu\text{g. per 100 ml.})} \times 100 = \text{ml. plasma cleared of steroid per min.}$$

Endogenous creatinine clearances were simultaneously performed in all experiments (Table 1).

RESULTS

The renal clearance of androsterone (expressed as milliliters of blood per minute) under all conditions studied was much greater than that of dehydroepiandrosterone. In Figure 1 are shown representative results for the clearance of dehydroepiandrosterone, following the oral administration of 2.0 Gm. of the acetate and of androsterone (free and hemisuccinate) at two dosage levels. These data were obtained in 3 separate experiments performed at one-week intervals. The highest blood levels of androsterone were achieved after the oral administration of 1.5 Gm. of its hemisuccinate

TABLE 1

Compound	Blood level (μg./100 ml.)	Renal clearance of steroid	
		ml. blood cleared/min.	% endogenous creatinine clearance
Dehydroepiandrosterone			
Control	45.6	2.2	2.4
Hemisuccinate i.v.	145	2.8	2.0
	159	2.0	2.4
Acetate	905	1.1	0.9
(2.0 Gm. orally)	941	1.1	1.0
	870	1.3	1.1
Androsterone			
Control	11.1	70.0	77.0
Hemisuccinate i.v.	579	24.9	17.5
	590	21.7	23.5
Free	491	31.7	20.5
(0.5 Gm. orally)	435	33.0	20.0
	410	50.8	37.0
Free	437	36	25.0
(2.0 Gm. orally)	426	59	36.5
	966	26.0	18.0
Hemisuccinate	4214	5.1	3.9
(1.6 Gm. orally)	3845	8.8	7.5
	3740	13.5	11.6
	4245	11.0	8.6

The data are grouped according to intervals of twenty-five minutes following the administration of the compound as detailed in the text.

in alkaline solution. Several experiments following the oral administration of androsterone revealed clearances which varied inversely with the blood levels; the results of numerous observations are plotted in Figure 2 and are contrasted with the clearances of dehydroepiandrosterone in the single experiment performed with the latter substance. The initial determinations at the lowest blood levels were made under basal conditions, utilizing the method of paper chromatography described previously. Otherwise the results in Figures 1 and 2 were obtained by the Zimmermann reaction

on the beta fraction for dehydroepiandrosterone and by the Pincus reaction on the alpha fraction. Comparison with other methods employed (namely, the Zimmermann reaction on both fractions, or the Allen blue reaction on the beta fraction) revealed clearances which were similar to those illustrated (within 10 per cent).

Repetition of the analyses on duplicate aliquots, by hydrolysis with glucuronidase after the administration of androsterone, and dioxane after dehydroepiandrosterone, indicated that all the androsterone was excreted as a glucuronoside and the dehydroepiandrosterone as the sulfate.

By examination of Figure 2 and Table 1, it may be seen that the clearances of androsterone, which were very high at the lower blood levels (70 ml. per minute), fell as the blood levels increased. The curve resembles that of the clearance of substances which are secreted by tubular mechanisms rather than glomerular filtration (17).

In a single instance the renal clearance of dehydroepiandrosterone was determined in a 5-year-old girl with adrenocortical tumor, who was excreting 21.2 mg. of 17-ketosteroids per day, 6.1 mg. of which were measured

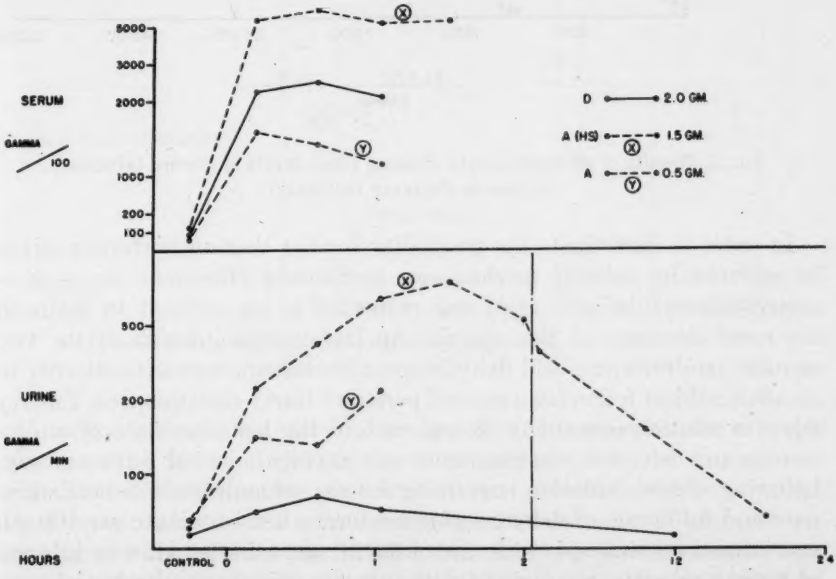

FIG. 1. Blood levels and urinary excretions of steroids after the administration of 2.0 Gm. dehydroepiandrosterone acetate (D), 0.5 Gm. of free androsterone (A), and 1.5 Gm. of androsterone hemisuccinate (A-HS) by mouth. Results are shown for 3 periods of twenty-five minutes each, up to two hours after administration of the steroid. (The data are translated into ml. of plasma cleared per minute in Table 1.)

as dehydroepiandrosterone. The plasma level of dehydroepiandrosterone was 598 micrograms per 100 ml. The renal clearance of dehydroepiandrosterone in this single patient was very low (2.5 ml. per minute) and consistent with the results for the renal clearance of dehydroepiandrosterone in a normal subject.

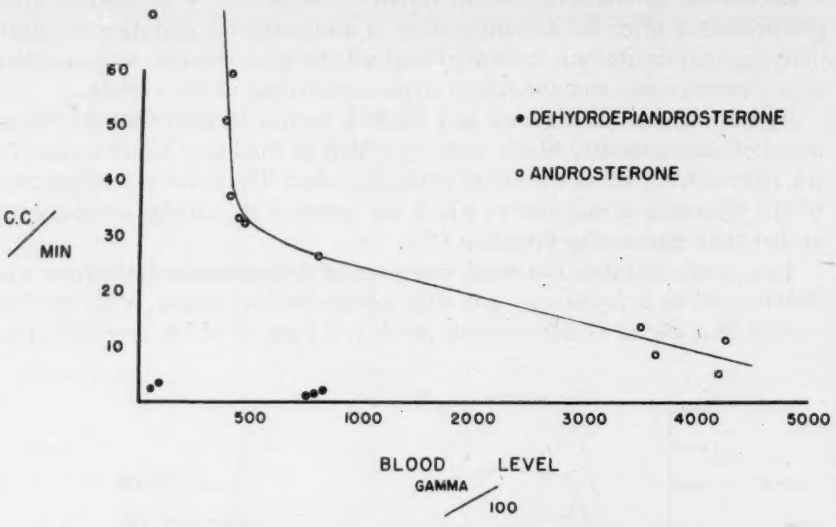

FIG. 2. Results of all experiments, relating blood levels of steroid (abscissa) to plasma clearance (ordinate).

In order to investigate the possibility further that androsterone might be secreted by tubular mechanisms, probenecid (Benemid; or *p*-(di-*n*-propylsulfamyl)-benzoic acid) was employed in an attempt to diminish the renal clearance of this steroid. An intravenous infusion of the two steroids (androsterone and dehydroepiandrosterone) was administered to an adult subject following a control period of thirty-nine minutes. Twenty ml. of a solution containing 78 mg. each of the hemisuccinate of androsterone and dehydroepiandrosterone was rapidly injected intravenously, following which a solution containing 8.3 mg. of androsterone hemisucicnate and 0.139 mg. of dehydroepiandrosterone hemisuccinate per 100 ml. was infused continuously at a rate of 5.5 ml. per minute. After an interval of twenty minutes, six periods of twenty-five minutes each were chosen, and separate urine specimens were collected for each interval. Blood was obtained at the midpoint of the first two and the last two periods. Immediately upon the termination of the second period, a dose of 2.5 Gm. of probenecid was administered by mouth. The results of this study are il-

lustrated in Figure 3, based on the same methods employed in Figures 1 and 2. It may be seen that the renal clearance of dehydroepiandrosterone was low throughout the experiment and was not increased following the administration of probenecid (Benemid); a small decrease occurred following probenecid, but the significance is questionable because of the low initial rate of excretion. However, the absolute quantity and the renal clearance of androsterone were markedly depressed by the administration

TABLE 2

Compound (hemisuccinate i.v.)	Period	Renal clearance (ml. plasma/minute)	
		A*	B*
Androsterone	Control		70.0
	1	24.9	24.0
	2	21.7	20.0
	Probenecid		
	5	2.7	3.2
	6	2.5	3.8
Dehydroepiandrosterone	Control		2.2
	1	2.8	3.4
	2	2.0	—
	Probenecid		
	5	0.6	1.6
	6	0.4	1.0

* A = Florosil chromatography and digitonin separation.
B = Paper chromatography.

of probenecid, and the level of androsterone in the plasma rose, as would be expected if the renal excretion were suppressed.

In Table 2 the clearances of each compound obtained during continuous intravenous administration are compared on the basis of methods employing chromatography on Florosil and digitonin fractionation versus those employing paper chromatography. A good correlation was obtained. Portions of the residues of several plasma specimens were pooled following chromatography on Florosil and digitonin precipitation, preparatory to the quantitative estimations described previously and were submitted to infrared spectroscopy through the kindness of Doctor Seymour Lieber-

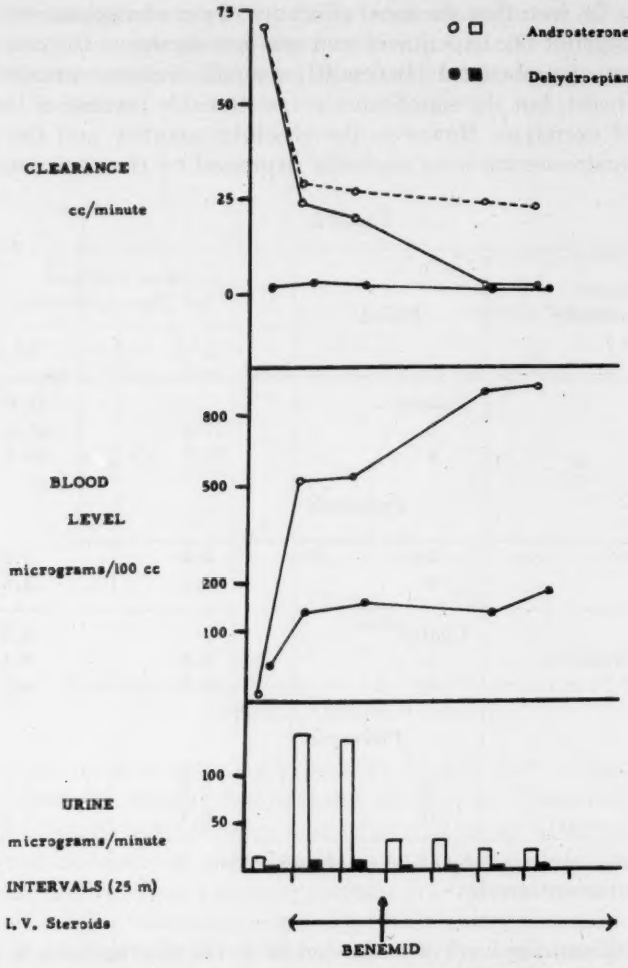

FIG. 3. Plasma volume cleared per minute, blood levels of steroids, and excretion of steroids in urine after the administration of the hemisuccinates of androsterone and dehydroepiandrosterone together by vein. The first values plotted are basal levels. The effect of probenecid (Benemid) administration is shown. In the upper portion of the graph the observed renal clearance of androsterone is shown by a solid line with open circles; the dotted line represents the theoretically expected clearance at each blood level estimated from the data in Figure 2. The difference indicates the depression by probenecid. The clearance of dehydroepiandrosterone is indicated by the solid lines with dots.

man. In each instance the spectrum was that of the steroid which had been administered by mouth (androsterone or dehydroepiandrosterone). The fractions isolated by paper chromatography were not further identified except according to their sulfuric-acid chromogens, which were identical with those of the expected compound.

A preliminary attempt was made to study the protein-binding of the two substances in human plasma. Untreated plasma, obtained after the oral administration of either compound during these experiments, was dialyzed against water and it was found that androsterone traversed the membranes to the extent of 50–76 per cent of its total blood level, and dehydroepiandrosterone not at all. Following zinc hydroxide precipitation of plasma samples, 42–60 per cent of androsterone appeared in the filtrate, whereas only 7–35 per cent of dehydroepiandrosterone appeared. Using precipitation with trichloracetic acid, 45 per cent of the dehydroepiandrosterone appeared in the filtrate, and 97 per cent of the androsterone. When the plasma was precipitated by heating at 50° C. for twenty minutes at pH 3.0, 28 per cent of the dehydroepiandrosterone and 100 per cent of the androsterone were present in the filtrate. When ammonium sulfate was employed as the precipitant in a saturated solution, neither androsterone nor dehydroepiandrosterone appeared in the filtrate. All plasmas used in these studies were obtained following the oral administration of either compound alone. No studies were carried out with human plasma under basal conditions. Large pools of red cells from the plasma specimens were frozen and thawed and the samples were treated as described, with the use of 70%-ethanol/petroleum-ether partition to remove the hemoglobin. Less than 1 per cent of either steroid, as related to the total plasma level, was found in the red cells.

In Table 1 all results are expressed as clearances of either compound related to creatinine.

In a single preliminary experiment, the renal clearance of beta-cortolone (pregnane-3α,17α,20β,21-tetrol-11-one) was determined. A normal adult human subject received 2 Gm. of this substance by mouth at intervals of thirty minutes for 8 doses, following which three periods of twenty-five minutes each were employed in order to determine the renal clearance. Utilizing an approach previously described (15) and measuring the free material in blood and urine by periodate oxidation, the clearances varied from 17.7 to 40.5 ml. per minute at blood levels of 942–1751 micrograms (μg.) per 100 ml. These clearances also varied inversely with the blood levels, suggesting tubular secretion. Curiously, the level of corticoids in the blood measured as Porter-Silber chromogens was 23.6 μg. per 100 ml. before the administration of cortolone, and fell to 0–0.7 μg. per 100 ml.

after its administration. The plasma 17-ketosteroid concentration remained unaffected.

The creatinine clearances averaged 133 ml. per minute in all the studies, and varied by less than 10.0 per cent of the mean in any single series.

DISCUSSION

The highest concentrations of steroids in the plasma after oral administration, followed the use of androsterone hemisuccinate, which is soluble in a weakly alkaline aqueous solution. The lowest level was seen after administration of dehydroepiandrosterone acetate. Such factors as the intestinal absorption of the various forms were not further studied. However, such variables have no bearing on the results, since the essence of the investigation lies in the relative concentrations of the compound in urine and plasma within a narrow interval of time.

The methods used during most of the studies yielded results which compared favorably with those obtained upon separation of the steroids by paper chromatography (Table 2). No doubt the similarity in this study may be attributed to the high concentration of steroids achieved after their administration, which eliminated the necessity for more elaborate techniques in accurate determination.

The results indicate that the preponderance of dehydroepiandrosterone in human plasma may be attributed to its low renal clearance. The high basal renal clearance of androsterone explains its low concentration in human plasma. Therefore, it may be concluded that, in man, the production of androsterone exceeds that of dehydroepiandrosterone, and that the high blood levels of the latter are secondary to the very low clearance of this steroid. Although other routes for the disposition of dehydroepiandrosterone may exist, these would not negate the proposed conclusion, since it was possible to maintain high blood levels of this compound during the continuous intravenous administration of a small dose approximating the small renal excretion (Fig. 3).

These studies in no way clarify the source of dehydroepiandrosterone in human blood, and some doubt has been cast on its origin from the adrenal cortex (4). It is probable that when the urinary concentration of dehydroepiandrosterone is very high (as in some instances of adrenocortical tumor) the blood levels may be extremely elevated—far in excess of the values observed in these experiments. Perhaps the difference in clearance between these two 17-ketosteroids is attributable to the difference in properties between the glucuronoside of androsterone and the sulfate of dehydroepiandrosterone. The clearance of androsterone resembles that of certain other glucuronosides which also appear to be secreted by renal tubular mech-

anisms (18). The different behaviors of the two steroids in human plasma upon dialysis or precipitation, although not considered conclusive, suggest that sulfates are more firmly bound to proteins than are glucuronosides. It is possible that under some circumstances either steroid may be excreted as a conjugate other than that ordinarily associated with it. By differential hydrolysis, it appeared that dehydroepiandrosterone occurred as a sulfate and androsterone as a glucuronoside in this study. Under certain conditions, dehydroepiandrosterone may be excreted as a glucuronoside or other conjugate which more readily escapes the kidney, but this has not been demonstrated.

The fact that the renal excretion of androsterone can be depressed by probenecid confirms the tubular secretion of this steroid, since probenecid is known to block the renal tubular secretion of compounds such as para-aminosalicylic acid and penicillin (19). Probenecid would not influence excretion if androsterone were predominantly eliminated by glomerular filtration. It is tenable that the renal clearance of dehydroepiandrosterone is low because of its rapid tubular reabsorption in the face of significant filtration by the glomerulus, in which case probenecid would have been expected to increase its excretion, as it does in the case of certain other substances such as uric acid, due to the inhibition of tubular reabsorption of filtered steroid (20). Since this did not occur, it seems that such a mechanism does not account for the low renal excretion of dehydroepiandrosterone, although the possibility that its reabsorption depends on tubular systems which remain unaffected by probenecid cannot be disclaimed. Gardner (21) had reported diminution of total 17-ketosteroids in urine following the administration of probenecid, although in his experiments there appeared to be interference with the excretion of both androsterone and dehydroepiandrosterone. In these studies it appears that only the excretion of androsterone is significantly affected by probenecid.

The brief study of the clearance of beta-cortolone is interesting, and suggests that this compound may also be secreted by the kidney tubules. Of particular interest is the apparent ability of beta-cortolone to depress the blood level of Porter-Silber chromogens. This deserves further investigation.

SUMMARY

The renal clearance of androsterone in man has been shown to be much greater than that of dehydroepiandrosterone. This explains the preponderance of dehydroepiandrosterone in human plasma on the basis of renal factors rather than on the basis of an inordinately high production of this steroid. The rapid renal clearance of androsterone, on the other hand, accounts for its greater concentration in human urine and indicates that its

over-all production is greater than that of dehydroepiandrosterone in man.

The renal excretion of androsterone is inversely proportional to the elevated blood levels produced by its administration, and is inhibited by probenecid; therefore, androsterone is eliminated by tubular secretion rather than glomerular filtration. It appears unlikely that dehydroepiandrosterone is reabsorbed by the renal tubules after significant glomerular filtration, since its urinary excretion is not enhanced by probenecid.

The renal clearance of beta-cortolone was also determined. Administration of this substance appeared to diminish the plasma levels of Porter-Silber chromogens in a single experiment.

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THE EXCRETION OF PREGNANE-3α,17α,21-TRIOL 20-ONE (TETRAHYDRO S) IN NORMAL AND PATHOLOGIC URINE

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PREGNANE-3α,17α,21-triol-20-one (tetrahydro S) has previously been identified in the urine of patients with adrenocortical tumors (1, 2) and in the urine of a man following the administration of corticotropin, by this laboratory (3). Eberlein and Bongiovanni (4) have found it in large quantities in the urine of a subject with adrenal hyperplasia. We have also identified it as the major alpha-ketolic metabolite in the urine following ingestion of Δ^4-pregnene-17α,21-diol-3,20-dione (Reichstein's compound S) by a patient with anterior pituitary failure (5). The following report describes the characterization of tetrahydro S and the situations under which this steroid was or was not found in urine during a more extensive series of observations.

Observations were made on pooled normal urine and on urine specimens from 2 normal subjects, 9 patients with hyperadrenocorticism, 1 with hypoadrenocorticism, and 8 with miscellaneous conditions, including pregnancy (Table 1).

MATERIALS AND METHODS

Methods. The methods employed and their limitations have been previously described in detail (5). Conjugates in the urine were hydrolyzed with β-glucuronidase at pH 4.5 for two days. The urine was then adjusted to pH 1 and extracted with chloroform for one hour on an oscillating shaker. Preliminary separation of fraction X (ref. 5) was made by developing the chromatograms in the toluene-propylene glycol system (6), collecting 15 cc. of effluent per ½-inch of strip (approximately ninety-six hours). Tetrahydro S migrates with cortisone in this system but is separated from hydrocortisone and other slower moving substances as well as the faster moving steroids. The zone containing the tetrahydro S and cortisone was eluted and the eluate rechromatographed in the benzene-formamide system which separated the two compounds well. After development of the chromatograms, the absorption at 245 mμ was determined directly in a Beckman DU spectrophotometer by means of an adaptor (7). Absorption at 600 mμ was similarly determined after spraying with blue tetrazolium to determine the location of alpha-ketolic compounds. Density was plotted against distance from the starting line. Semiquantitative estimates (±30 per cent) of the various alpha-ketolic steroids

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TABLE 1. URINARY EXCRETION OF MAJOR ALPHA-KETOLIC STEROIDS (X FRACTION)*

First day of collection	Subject: Inits.	Age (yrs.)	Sex	Diagnosis	H_4E* and H_4F (mg./24 hrs.)	F (mg./24 hrs.)	E position† (approx.)	H_4S	Identification of H_4S‡: a	b	c	d	e	f	17-KS (mg./24 hrs.)	G'ase R-L§
				NORMAL												
Sept. 19, '55	ST.	23	M	—	1.1‖	0.07	0.08	<¶	−¶	−	−	−	−	−	Normal range (M =6–25)	(6–20)
July 29, '55	DO.	48	M	—	1.3‖	<	<	<	−	−	−	−	−	−	(F =6–15)	(6–20)
1954	Pool	—	M&F	—	1.3	0.06	0.14	0.02	+¶	+	+	+	+	−	—	—
				ADRENOCORTICAL HYPERFUNCTION												
				a. *Adrenocorticotropin (10 mg. I.V. in 8 hrs.)*												
Oct. 19, '52	HI.♯	37	M	Normal (1st day)	2.7	1.2	0.41	0.26	+	+	+	+	+	−	26.2	25.7
Nov. 2, '52	ME.♯	23	F	Normal (1st day)	3.9	1.1	0.50	<	−	−	−	−	−	−	29.0	25.2
Mar. 8, '54	OU.♯	26	M	Rheum. fever (5 days)	2.2	1.4	0.20	0.46	+	+	+	+	+	+	13.2	27.7
				b. *Without Adrenocortical Tumor*												
Aug. 7, '54	RUT.	20	F	Cushing's syndrome	2.5	0.8	0.42	0.25	+	+	−	−	−	−	—	16.6
May 3, '52	MUS.♯	19	M	Cushing's syndrome	5.3	1.0	1.2	1.7	+	+	+	+	+	−	23.6	38.6
				c. *With Adrenocortical Tumor*												
Aug. 17, '55	BO.	26	F	Cush. syn. (adenoma)	9.1‖	1.1	0.39	<	−	−	−	−	−	−	7.2	47.3
Apr. 7, '54	SCH.	43	F	Cush. syn. (metast.)	5.0	1.1	0.76	15.0	+	+	+	+	+	+§	22.9	83.7
Aug. 12, '54	SA.♯	37	M	Feminizing (metast.)	2.7	0.17	0.19	2.5	+	+	+	+	+	+	—	32.1
Oct. 10, '50	CA.♯	48	F	Virilizing (metast.)	1.1	<	<	12.4	+	+	+	+	+	+	—	64.0
				ADRENOCORTICAL HYPOFUNCTION												
Nov. 25, '51	AL.♯	30	F	Ant. pit. failure	<	<	<	<	−	−	−	−	−	−	2.0	3.2
				MISCELLANEOUS CONDITIONS												
Jan. 9, '54	BU.	58	M	Essential hypertension	0.75	0.14	0.21	0.07	+	+	+	+	−	−	7.8	20.3
Feb. 9, '54	DIR.	13	F	Essential hypertension	1.1	—	<	<	−	−	−	−	−	−	—	8.6
Oct. 30, '53	RO.	34	F	Essential hypertension	1.2	0.07	0.09	<	−	−	−	−	−	−	—	8.0
Sept. 19, '54	MCS.	37	F	Diabetic retinopathy	1.3	0.04	0.09	<	−	−	−	−	−	−	—	13.4
Oct. 23, '54	RUB.	58	F	Diabetic retinopathy	1.7	—	0.03	<	−	−	−	−	−	−	—	—
Oct. 10, '53	US.	28	F	Pregnancy (2nd mo.)	0.55	<	0.13	<	−	−	−	−	−	−	—	—
Feb. 18, '54	HA.	25	F	Pregnancy (pooled urine)	1.4	—	0.14	<	−	−	−	−	−	−	—	—
Dec. 19, '54	BL.	43	M	"Potassium wasting"	1.3	<	0.07	<	−	−	−	−	−	−	—	—

* Since cortisone and hydrocortisone (compounds E and F) and their tetrahydro (H_4) forms have been previously isolated and characterized, the complete identification of these compounds was not attempted. In some instances, the identification procedures included spectrometry in sulfuric acid in addition to routine observation of chromatographic characteristics, blue tetrazolium reaction, and ultraviolet absorption. The X fraction contains the more polar compounds (*see* Methods).

† The term "E position" is used, since rechromatography of some extracts demonstrated 0 to 4 unidentified blue tetrazolium reducing zones with the same mobility as cortisone in the benzeneformamide system (*see* text).

‡ Identification procedures for tetrahydro S (H_4S), in addition to reaction with blue tetrazolium and lack of absorption of ultraviolet light.

a. Rate in toluene/propylene-glycol system
b. Rate in benzene/formamide system
c. Rate of acetate in methylcyclohexane/propylene-glycol system
d. Porter-Silber reaction (for 17-hydroxylation)
e. Absorption spectrum in sulfuric acid
f. Infrared spectrometry (courtesy of Dr. Thomas F. Gallagher)
g. Melting point also determined (*see* text).

§ G'ase. R-L = Neutral reducing lipids after glucuronidase and acid hydrolysis.

‖ Extracted by an improved technic. Yields were approximately 50 per cent greater.

¶ Symbols:
< Not detected at level examined. In the case of tetrahydro S and compound E this indicates a minimum of 20 μg. per day, as rechromatography was carried out (*see* text). In the case of the other metabolites the minimum required for semiquantitative estimation was 100 μg. per day unless rechromatography was performed using larger aliquots.
− Not determined.
+ Agreed with reference steroid under equivalent conditions.

♯ Some of these results and some clinical data have been previously reported in part for Subjects HI., ME., MU. (21); CA. (1, 21, 22); OU. (2); AL. (5); and SA. (22).

were made by planimetric determinations of the areas of the peaks (5). Neutral reducing lipid-soluble substances were determined by the method of Heard, Sobel and Venning (8), after glucuronidase hydrolysis as previously described (5). The total neutral 17-ketosteroids were determined by a modification of the method of Holtorff and Koch (9).

Identification of tetrahydro S. The isolated tetrahydro S in all cases was identified by mobility characteristics, both as the free compound and as the acetate in various systems, as indicated in Table 1. The quantitative determination of 17-hydroxylated alpha-ketolic steroids by the Porter and Silber technic (10) yielded a value that agreed with the amount of alpha-ketolic steroid calculated from the degree of reaction with blue tetrazolium. There was no absorption of light in the ultraviolet zone at 245 mμ. Spectrometry in sulfuric acid showed a spectrum with characteristic peaks at 315 and 410 mμ as was shown by the authentic sample. In 4 instances (see Table 1) infrared spectrometry (courtesy of Dr. Thomas F. Gallagher) substantiated the identification. Table 1 shows the extent of identification of the isolated tetrahydro S in each case studied. Sulfuric-acid spectral studies and infrared spectrometry were performed only in those instances in which sufficient material was available.

Crystalline material obtained in Subject SCH. was purified (after elution from chromatograms) by recrystallization from alcohol—melting point 195–200°. This material was acetylated with pyridine and acetic anhydride to form the diacetate. The acetate melted at 196–203°, after chromatography on a Celite-silica gel 1:1 column and recrystallization from acetone-methylcyclohexane. The mixture melting point of the acetate combined with authentic material of 205–206° M.P. was not depressed. Rosselet *et al.* (2) reported a melting point of 201–206° for the diacetate. Ungar and co-workers (11, 12) reported 200–204° as the melting point for the free compound and 201–206° for the diacetate.

RESULTS

In Table 1 are listed the semiquantitative estimates of the major urinary alpha-ketolic compounds found in fraction X, namely, cortisone, hydrocortisone, and their tetrahydro derivatives, as well as tetrahydro S. The values for urinary 17-ketosteroids and neutral reducing lipids after glucuronidase hydrolysis are also listed for comparison.

In the 3 cases of adrenocortical carcinoma, the excretion of tetrahydro S was high, but this compound was not detected in the urine of a woman with an apparently non-malignant adrenocortical tumor producing Cushing's syndrome. In the 2 patients with Cushing's syndrome without demonstrable tumor, large amounts of tetrahydro S were excreted by the man, and considerably less by the woman. In 5 of the 6 cases of pathologic adrenal hyperactivity there was an increased output of compound F and its metabolites; in the sixth (Subject CA.) the output was normal. However, the output of tetrahydro S in the patients with adrenal carcinoma accounted for 45–92 per cent of the alpha-ketolic metabolites seen in this chromatographic fraction.

Tetrahydro S was not detected in the urine of 2 normal men, although an improved extraction technic (providing approximately 50 per cent greater yield) was used in these 2 cases. The level of tetrahydro S in the "normal pool," which included urine from both men and women, was

shown to be about 20 micrograms per day. No tetrahydro S was found in the urine of 2 pregnant women nor in that of 2 women with diabetes complicated by severe retinopathy. In 1 man with hypertension there was a demonstrable amount of urinary tetrahydro S, but in 2 women with hypertension none was demonstrated. No detectable amounts were found in a woman with anterior pituitary failure, but after oral administration of compound S to the same individual large amounts were identified (5). The excretion of cortisone, hydrocortisone and their tetrahydro derivatives in the normal and miscellaneous groups was approximately of the same order as that reported by DeCourcy *et al.* (13) and by Cope and Hurlock (14).

The intravenous administration of adrenocorticotropic hormone to a normal man for one day and to a man with rheumatic fever for five days, at the rate of 10 mg. over eight hours, resulted in the appearance of identifiable amounts of tetrahydro S in the urine. However, a normal woman who was given adrenocorticotropic hormone in a similar fashion for one day failed to excrete detectable amounts of tetrahydro S. All those given corticotropin responded with a marked increase in the urinary excretion of hydrocortisone, cortisone and their tetrahydro derivatives.

DISCUSSION

Previous experiments (3, 5) indicated that tetrahydro S is the major alpha-ketolic steroid metabolite found in the urine after the administration of compound S. Ungar and Dorfman (12) have identified it in urinary extracts following administration of pregnane-17α,21-diol-3,20-dione (dihydro S). The logical mechanism for the large amounts of tetrahydro S found in the urine of the 3 patients with adrenal tumor would appear to be reduction in the liver of the compound S presumed to be produced by the adrenal tumor. However, incubation studies of adrenal tumor tissue from patients excreting large amounts of tetrahydro S failed to reveal evidence of compound S (15). The reasons for this are not clear. Tumor tissue may require different incubation conditions, since adrenal tissue removed from hypertensive patients demonstrated suggestive evidence of compound S production (16). Thus, compound S has not been shown to be an important product of the human adrenal nor has it been unequivocally identified in urinary extracts.

In this respect, it is of interest that compound S was not found in the urine after its administration to a patient with anterior pituitary failure (5). The five other "active" alpha-ketols were invariably found in the urine in appreciable amounts after oral administration of comparable doses. This may indicate that compound S is the most readily metabolized of the adrenal alpha-ketolic steroids.

In Cushing's syndrome, and in the subjects with adrenal tumors, there

was increased urinary excretion of cortisone and hydrocortisone and their tetrahydro metabolites, with the exception of Subject CA. This probably indicates an increased production of hydrocortisone, as this compound has been shown to be convertible to cortisone (5, 17, 18, 19). Considerable evidence indicates that compound S may be a precursor of hydrocortisone (20). However, in some cases of adrenocortical tumor there may be a relative lack of the 11-hydroxylating enzyme system. Since 11-hydroxylation probably follows 21-hydroxylation, one could expect a blocking or overloading of this system to lead to excretion of large quantities of compound S or its metabolite tetrahydro S.

SUMMARY

1. The identification of pregnane-3α,17α,21-triol-20-one (tetrahydro S) is discussed.

2. The urinary excretion of tetrahydro S has been studied by paper chromatographic methods. Urine was obtained from normal and pregnant subjects, from patients with hyperfunction or hypofunction of the adrenal cortex, and from patients with miscellaneous conditions. Excretion of tetrahydro S varied from "undetectable" to 15 mg. per twenty-four hours. The highest values were found in the patients with adrenocortical tumors.

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THE OCCURRENCE OF 3-ALPHA, 21-DIHYDROXY-PREGNANE, 11-20-DIONE (TETRAHYDRO COMPOUND A) IN THE SERUM OF CHILDREN*

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3-Alpha,17-alpha,21-trihydroxypregnane,11-20-dione (tetrahydro compound E) has been recovered from the conjugated corticoid fraction of plasma after the oral administration of this steroid to a human subject by Bongiovanni and Eberlein (1). In addition, these same authors have presumptively identified 3-alpha,17-alpha-21-trihydroxypregnane-20-one (tetrahydro S) in the conjugated corticoid fraction of the plasma of a patient with congenital adrenal hyperplasia (2). This identification was made by means of staining characteristics and the mobility during paper chromatography. The present report is the result of an attempt to identify the conjugated corticoids in human serum after the administration of corticotropin.

MATERIALS AND METHODS

Serum samples were obtained from children 5 to 14 years of age, two hours after the intravenous administration of 10 mg. of corticotropin (ACTH) per square meter of surface area. The injection was given within two minutes in a volume of 2–4 ml. of physiologic sodium chloride. The sera so obtained were pooled, so that each analysis was made on a volume of approximately 150 ml., representing approximately 15 children. When the serum was to be divided and half the serum was to be hydrolyzed with glucuronidase and half with acid, a total aliquot of approximately 200 ml. was used. The children were recovering from various acute illnesses or were hospitalized for study of neurologic and convulsive disorders. None of them was considered to have any endocrine disease, but otherwise no selection of patients was made. The serum was immediately frozen. When sufficient quantities were obtained, the samples were thawed and pooled. The free corticoids were removed by chloroform extraction or by the method of Bongiovanni (1). The conjugated corticoids were extracted with alcohol and then hydrolyzed

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either with beta-glucuronidase according to the method of Bongiovanni, or with hydrochloric acid. The extracts of the steroids were passed through a Florosil column according to the method of Nelson and Samuels (3). The corticoid extracts were chromatographed on paper using the solvent system C of Bush (4). A thin vertical portion of the paper strip was stained with blue tetrazolium or triphenyl-tetrazolium hydrochloride. The steroids in the areas of paper corresponding to the stained corticoids were eluted with methanol. The methanol was then evaporated under an air blast. Concentrated sulfuric acid was added to the dry steroid and the sulfuric-acid chromogens were analyzed on a Beckman DK recording spectrophotometer by the method of Zaffaroni (5). In addition, other paper strips were sprayed with sodium hydroxide and examined for fluorescence under ultraviolet light by the method of Bush (6). Ultraviolet scanning was carried out on unstained paper strips and the absorption pattern of the steroid in alcohol in the ultraviolet area was also observed.

RESULTS

When serum from which the free corticoids had been removed was hydrolyzed with beta-glucuronidase, materials were found which were stainable with blue tetrazolium and triphenyl-tetrazolium. These materials had mobilities identical to those of standard tetrahydro F, tetrahydro E and tetrahydro A. Upon elution, the Zaffaroni chromogen results were typical of those obtained with standard compounds analyzed in similar fashion. None of the areas showed fluorescence with sodium hydroxide staining, nor could they be seen with ultraviolet scanning on the unstained paper. In addition, none of the specimens in alcohol had an absorption peak at 240 mμ.

Figure 1 shows the appearance of a typical paper chromatogram and indicates the staining and spectrophotometric characteristics of the 3 conjugated corticoids found in human blood after glucuronidase hydrolysis.

When the serum pools were divided, and half was hydrolyzed with hydrochloric acid and half with beta-glucuronidase, materials staining with blue tetrazolium and having the mobilities of tetrahydro F and tetrahydro E were found on the parallel papers. There seemed to be less of these materials, especially tetrahydro F, in the specimen hydrolyzed with acid. They were not further identified. No tetrahydro A was found in these smaller aliquots.

DISCUSSION

Absolute identification of these compounds has not been made because of the small quantities available. However, it is felt that the presumptive identification is strong and that the serum of children after administration of corticotropin contains the reduced derivatives of compounds E, F and A.

Bongiovanni was not able to find any identifiable steroid after acid hydrolysis of plasma, nor was he able to hydrolyze tetrahydro-E glucuronide

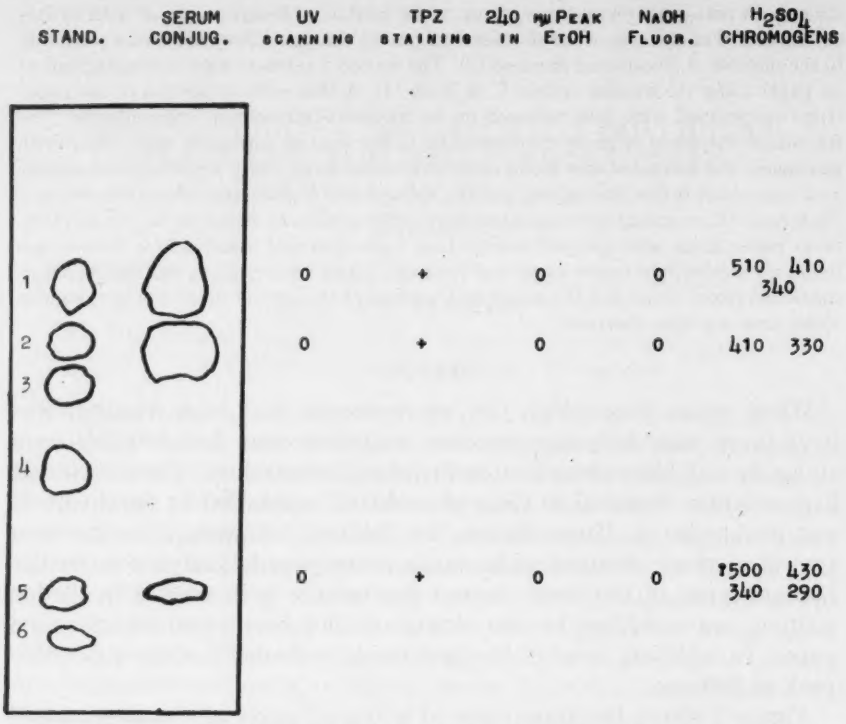

FIG. 1. Paper chromatogram stained with TPZ, and staining and spectrophotometric characteristics of conjugated corticoids of serum.

Standards: 1=tetrahydro F; 2=tetrahydro E; 3=compound F; 4=compound E; 5=tetrahydro A; and 6=compound A.

with acid (1). The reason for the discrepancy between our results and his is not immediately apparent but perhaps we can make some suggestions. The failure to find analyzable corticoids in plasma hydrolyzed with hydrochloric acid may be due to the quantity of plasma employed. The acid destroys approximately 50 per cent of the derivatives of compound F, though the derivatives of compound E are relatively stable under this procedure. This hypothesis is further supported by our impression (which admittedly is a rough subjective one) that there was less tetrahydro F in the aliquots hydrolyzed with acid than in equal aliquots hydrolyzed with beta-glucuronidase. Bongiovanni has suggested that the Porter-Silber chromogens after acid hydrolysis were nonspecific artifacts. It is also possible that they were not artifacts, but that they existed in a quantity which was insufficient to be analyzed and identified by the method used. Furthermore if these materials are nonspecific chromogens, it is difficult to

explain the fact that their levels seem to rise and fall in the serum in a manner similar to that of the chromogens released by beta-glucuronidase both in health and disease. The curves of their serum levels and the curves of the levels for the beta-glucuronidase hydrolyzed corticoids obtained independently by Bongiovanni seem to be very similar (7–9). If steroid glucuronides in the blood cannot be hydrolyzed by acid, the only explanation we can offer is that part of the reduced corticoids may be conjugated with glucuronic acid and an equivalent part with some other substance—one conjugate being hydrolyzed by acid and the other conjugate by beta-glucuronidase. We have no evidence of this. However, the quantitative values for conjugated corticoids estimated by the Porter-Silber color reaction have been found to be much higher after butanol extraction, than after ethanol extraction, followed by similar hydrolytic methods (10). These findings with butanol have been irregular, however, and their significance is unclear, just as is the significance of the results when urine is extracted with butanol in similar fashion. Nevertheless, they suggest that not all the corticoid conjugates are released by the present methods.

The findings of tetrahydro compound A in the serum of human subjects after hydrolysis with beta-glucuronidase is not unexpected, though it has not before been described.

SUMMARY

A study has been made of the occurrence of tetrahydro compound A in the serum of children after intravenous administration of corticotropin. Samples of serum were pooled, so that a 150-ml. aliquot represented the sera of 15 children. Such aliquots were hydrolyzed with beta-glucuronidase and with acid after the free corticoids had been removed. Presumptive identification has been made of the conjugated corticoids, utilizing the mobility of the compounds during paper chromatography, their staining characteristics, and results with the Zaffaroni sulfuric-acid chromogen technique. Identified in this manner among the corticoids released by beta-glucuronidase hydrolysis have been 3-alpha,17-alpha,21-trihydroxypregnane,11-20-dione (tetrahydro compound E), 3-alpha,11-beta,17-alpha,21-tetrahydroxypregnane, 20-one (tetrahydro compound F) and, for the first time, 3-alpha,21-dihydroxypregnane,11-20-dione (tetrahydro compound A). When acid hydrolysis was used, smaller amounts of tetrahydro E and F were identifiable, and only by their mobility on paper and their staining characteristics.

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STUDIES ON ENDOMETRIAL GLYCOGEN

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THIS paper embodies the continuation of our studies on endometrial glycogen, based primarily on the application of the methods of Petersen and Rose using anthrone (1). A previous paper by Sabin and Latour (2) described the application of this reagent with definite color reaction as a qualitative test for detection of secretory-phase endometrium. A second paper by Payne and Latour (3) described the application of the photoelectric quantitative measurements of this color reaction to the estimation of glycogen levels in normal endometrium during the cyclic changes, as well as in the abnormal endometrium.

The main purpose of this paper is to report the continuation of the application of the quantitative measurements in cyclic normal endometrium, in order to validate the physiologic curve presented by Payne and Latour (3), which was based on a relatively small number of patients. The second purpose is to stimulate interest and discussions on the possible purpose of glycogen in the endometrium and to determine its relationship to implantation.

The quantitative anthrone method of Petersen and Rose was adopted, because it has definite advantages over the methods used by other workers (4–7). It is the simplest and most rapid procedure, and at the same time is more accurate and yields the highest degree of recovery. The main advantage is that it allows elimination of protein material and other contaminants to a degree not possible in previous methods. Most of these older procedures necessitated the neutralization of the digest in alcohol so that both glycogen and other proteose materials were precipitated. The latter were subsequently destroyed by acid hydrolysis, and this was the part of the procedure which caused unsatisfactory results (7). Anthrone reagent eliminates the lengthy step of hydrolysis entirely.

METHOD

The main steps of this procedure are as follows:

1. Precipitation of proteose materials by adjusting the pH to 4.0 in the absence of alcohol.

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2. Precipitation of glycogen, which occurs immediately upon the addition of alcohol.
3. Photoelectric colorimetry, using the anthrone reagent.

For this study, a few minor modifications were made in the method of Petersen and Rose. These consisted of shortening the time of exposure to air from the moment the wet tissue was obtained until immersion in potassium hydroxide solution, so as to avoid glycogenolysis as much as possible. This permitted a slight increase in the degree of recovery in almost all specimens, as shown in Table 1. It was found that a longer period

TABLE 1

Serial No.	Glycogen content of tissue (Gm. %) when exposed to air		Amount of increased recovery with 5 min. or less exposure
	for 10–13 min.	for 5 min. or less	
27	0.17	0.17	±0
24	0.19	0.20	+0.01
29	0.22	0.20	−0.02
9	0.25	0.25	±0
3	0.27	0.28	+0.01
8	0.35	0.37	+0.02
14	0.52	0.54	+0.02
18	0.52	0.59	+0.07
6	0.72	0.77	+0.05
11	0.80	0.84	+0.04
19	1.01	1.09	+0.08

of refrigeration as well as longer centrifugation during the alcohol precipitation of the glycogen helps to separate the phases more completely. The recovery possible with this modified Petersen and Rose procedure is much higher than that with other methods, as shown in Table 2.

Our endometrial specimens were dated according to the method of Hertig and Rock. Our dating coincided with the clinical dating in 72.2 per cent of the cases (allowing an error of ±2 days), as shown in Table 3.

Our specimens were stained for glycogen using periodic acid-Schiff (PAS) reagent (8–11). Although other staining methods produce good results, we felt that one of the simpler modifications of the PAS method was satisfactory for our purpose. Buffered alcoholic solutions, as suggested by Pearse (8), were not used; neither were collodionized sections (8). The fixative was a mixture of alcohol saturated with picric acid and formalin, as recommended by Deane, Nesbett and Hastings (12) and by Lison and Vokaer (13) in order to prevent polarization of the glycogen to one pole of the cell or diffusion into the fixing fluid. This method furnishes a better impression of glycogen distribution in living tissue. Corresponding slides were treated with a tested sputum to remove the glycogen by salivary digestion; this allowed the identification of pure glycogen by comparing the slide pairs.

TABLE 2. COMPARISON OF AVERAGE GLYCOGEN ESTIMATIONS OBTAINED BY DIFFERENT WORKERS AT VARIOUS STAGES OF THE MENSTRUAL CYCLE

(Glycogen values in Gm. %)

Workers	Proliferative phase		D (16) D (17) D (18) ("early differentiative")		Secretory phase	
Zondek and Stein	0.1 (4 specimens)		0.61 (2 specimens)		0.36 (11 specimens)	
Randall and Power	0.18 (4 specimens)		0.74 (8 specimens)		0.72 (18 specimens)	
Payne and Latour	Age group 20–35	36–45	Age group 20–35	36–45	Age group 20–35	36–45
	0.22 (6 specimens)	0.25 (5 specimens)	0.92 (8 specimens)	0.95 (8 specimens)	0.6 (22 specimens)	0.74 (17 specimens)
Arronet and Latour	0.35 (12 specimens)	0.28 (13 specimens)	1.2 (18 specimens)	1.05 (14 specimens)	0.56 (20 specimens)	0.72 (19 specimens)
	(+)0.013	(+)0.03	(+)0.18	(+)0.1	(−)0.04	(−)0.02

TABLE 3. RELATIONSHIP BETWEEN HISTOLOGIC AND CLINICAL DATING OF ENDOMETRIUM IN NORMAL OVULATORY CYCLES*

No. of cases	Histologic dating	Range of clinical dating	Coincidence of clin. with hist. dating (±2 days)	Percentage of coincidental dating
15	D (16)	D (11)–D (28)	9	60
10	D (17)	D (11)–D (28)	5	50
4	D (18)	D (16)–D (27)	3	75
4	D (19)	D (15)–D (20)	3	75
5	D (20)	D (15)–D (28)	3	60
10	D (21–22)	D (20)–D (28)	7	70
3	D (23)	D (22)–D (25)	3	100
3	D (24)	D (19)–D (25)	2	66
4	D (25)	D (16)–D (30)	2	50
5	D (26)	D (24)–D (28)	3	60
6	D (27)	D (18)–D (28)	4	66
3	D (28)	D (23)–D (1)	1	33
62			*45*	*72.2%*

* Menstrual cycles ranged from 26 to 30 days.

RESULTS

In this series, *quantitative* glycogen determinations were made on 97 specimens of normal endometrium. The women were divided into two age

groups (20–35 and 36–45 years) and an attempt was made to differentiate between "early" and "late" proliferative endometrium in the younger age group. From the scatter diagrams (Figs. 1 and 2) it may be seen that there was no significant difference in glycogen levels between the two age groups. The striking feature was the very high values obtained on day 17 of the cycle—one specimen reaching 2.1 Gm. per 100 Gm.

Since the primary purpose of this paper is to validate the results obtained by Payne and Latour (3) on normal endometria showing an un-

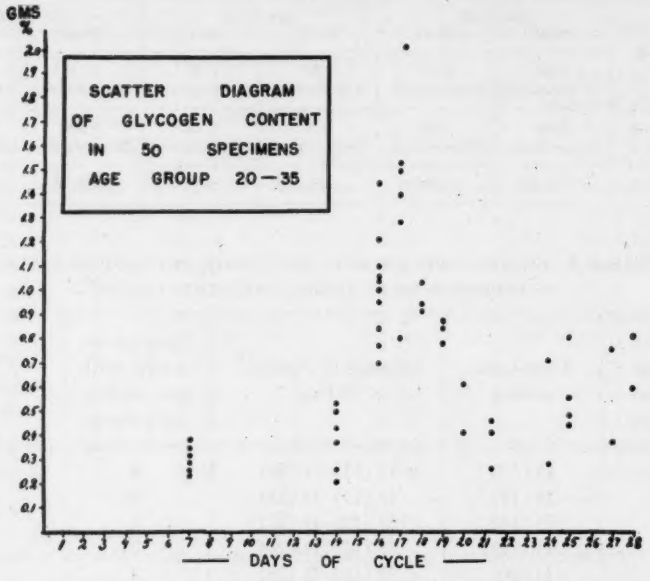

FIGURE 1

expected peak of glycogen concentration at day 17, and since the methods used and the results obtained in this series of 97 normal specimens are similar to those of Payne and Latour, we felt justified in superimposing the two groups of cases, as shown in the combined scatter diagram (Fig. 3). The probable physiologic curve (Fig. 4) derived from these cases is very similar to the curve of Payne and Latour based on the smaller series, and shows a slight gradual rise of glycogen during the proliferative phase followed by a definite and pronounced rise on the seventeenth day of the cycle. This rise is followed by a dip at twenty-one days and the level rises again and is maintained for the remainder of the cycle.

During this study the *qualitative* test of Sabin and Latour (2) was applied to 23 of the specimens. We were impressed by the fact that the de-

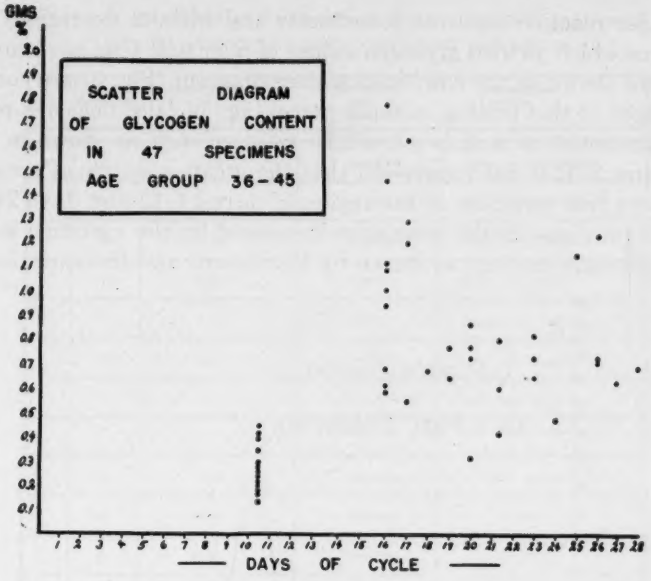

FIGURE 2

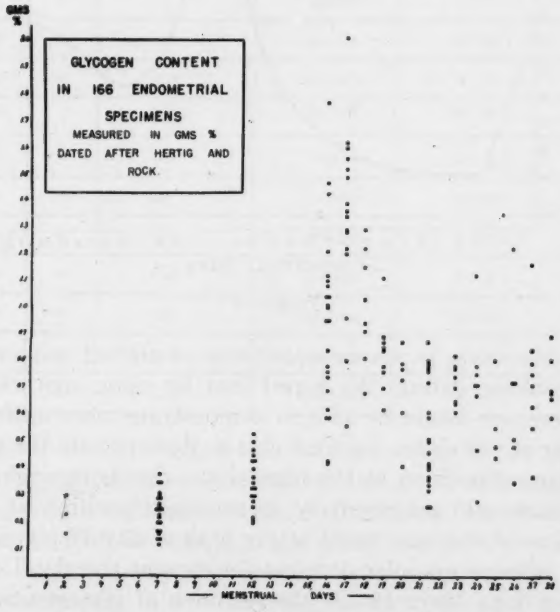

FIGURE 3

cisive color reaction occurred (constantly and without exception) only in specimens which yielded glycogen values of over 0.35 Gm. per cent. When the values shown in the combined scatter diagram (Fig. 3) are considered in the light of this finding, a small percentage of false negative reactions can be predicted, as well as a few false positive ones, as shown in Table 4 and Figure 5. It is our impression that the greatest source of error in the qualitative test would be in the region of days 14–15 and days 21–22.

In our previous studies were were impressed by the variation in the extent of glycogen content as shown by PAS stains and the variation in the

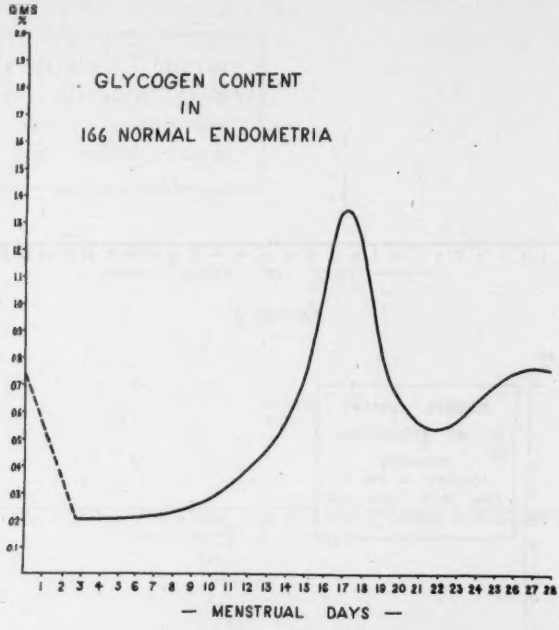

FIGURE 4

sites of its deposition in several specimens of normal endometrium with identical histologic dating. We hoped that by using another fixative, as described here, we would be able to demonstrate more uniformity. In a large number of our slides, we were able to demonstrate the tendency for slight glycogen deposition at the base of the glands throughout the proliferative phase, and progressively increasing deposition at the luminal edge as well as at the base, until at the peak of day 17 glycogen is found in the form of large granular droplets throughout the gland cells. During the following days (days 18–23) the secretion of glycogen appears to be initiated by an increased intracellular pressure or an increase in the

TABLE 4. QUANTITATIVE AND QUALITATIVE GLYCOGEN VALUES OF PRE-OVULATORY AND POST-OVULATORY ENDOMETRIA

Serial No		Quantitative test	Qualitative test	Endometrial dating
1	81	0.19	negative	Early proliferative
2	104	0.25	negative	Early proliferative
3	102	0.35	negative	Early proliferative
4	88	0.35	negative	Early proliferative
5	99	0.37	positive	Early proliferative
6	89	0.42	positive	Late proliferative
7	94	0.43	positive	Late proliferative
8	47	0.45	positive	Late proliferative
9	74	0.321	negative	D (20)
10	105	0.46	positive	D (22)
11	95	0.56	positive	D (24)
12	82	0.56	positive	D (17)
13	72	0.6	positive	D (22)
14	69	0.61	positive	D (20)
15	103	0.69	positive	D (28)
16	75	0.69	positive	D (16)
17	55	0.78	positive	D (19)
18	78	0.8	positive	menstrual
19	92	0.82	positive	D (26)
20	96	1.12	positive	D (16)
21	101	1.21	positive	D (16)
22	97	1.29	positive	D (17)
23	91	1.39	positive	D (17)

permeability of the cell wall, which causes leakage of the glycogen from any portion of the cell. Leakage between the gland cells can be observed, as well as back into the stroma of the endometrium. However, most of the glycogen finally finds its way into the gland lumen. We were not able to demonstrate satisfactorily the gradual progression of the intact basal vacuole to the luminal border in order to discharge its glycogen content into the lumen of the gland.

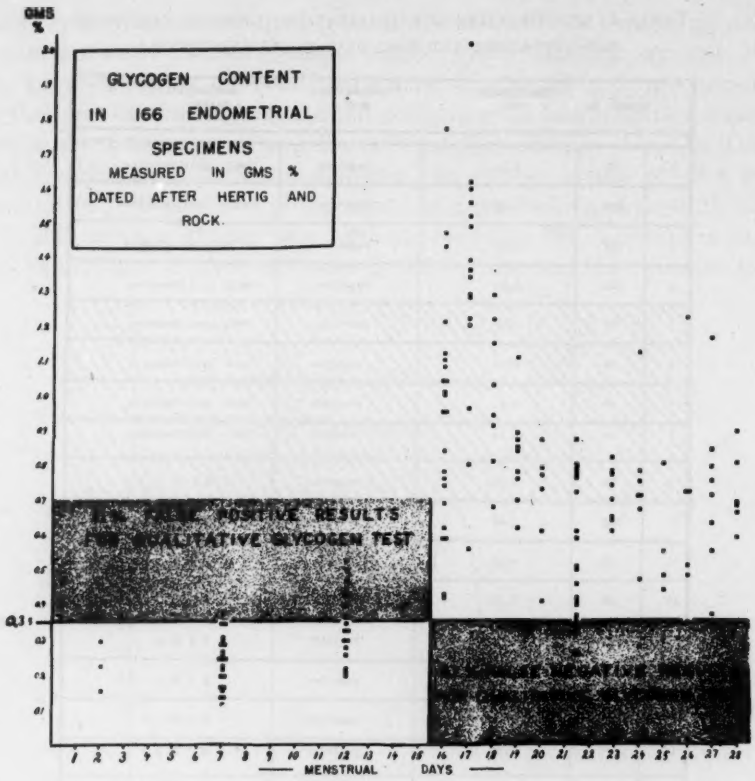

FIGURE 5

Our present studies reinforced our previous opinion that there is not a good correlation between the values obtained by quantitative measurements of glycogen by the Petersen and Rose anthrone method and the density and distribution of this material as demonstrated by PAS stains. Therfore it would seem that measurement of the glycogen content of endometrium by staining methods is unreliable and should be abandoned in favor of direct quantitative determinations. Furthermore, our observations point to the fact that the appearance of glycogen in the endometrium is the result of a complex phenomenon and represents perhaps an intermediate metabolic process within the endometrium and the structures functionally linked to it. The participation of ribonucleic acids in secretion, the involvement of other carbohydrate and protein fractions, and the fact that the secretory process is dependent on oxidation and utilization of glycolytic metabolites, point to the complexity of glycogenesis and glycogenolysis. These two processes appear to be taking place simul-

taneously at different sites in the endometrium. It is also apparent that the glycogen present in the glandular epithelium is converted to saliva-resistant poly- or mono-saccharides in the gland lumen. Further consideration along these lines is not within the scope of this paper.

An attempt was made to correlate morphologically abnormal endometria with glycogen concentration values. The impressions obtained from this effort were as follows:

1. In cases of endometrial hyperplasia the glycogen values are within the range for the normal proliferative phase.
2. In cases of morphologic deviation from the classic pictures of proliferative, secretory or premenstrual phases, called atypical rather than pathologic, the glycogen levels approximate those of the typical phase. These minor deviations do not appear to interfere with the function of the endometrium, at least insofar as glycogenesis is concerned.
3. In specimens showing chronic endometritis, the concentration of glycogen was very low.
4. A few specimens from patients receiving testosterone or thyroid therapy (in some cases long after the menopause) had unusually high glycogen contents.
5. Specimens taken from 18 infertile women who had no gross pathology in the genital tract or systemic disease were examined over an average observation time of five months, with the following results: a) In 9 patients there was evidence of normal secretory endometrium and corresponding normal glycogen concentration. Unfortunately 5 of these patients did not reappear at the sterility clinic, so there were no further observations. Of the remainder, 3 became pregnant. b) In 4 patients having various endometrial conditions as listed in sections 1 and 3, there were corresponding deviations of glycogen concentration in the endometrium. None of them became pregnant. c) In 2 patients having normal secretory endometrium there were abnormally high concentrations of glycogen. One of these women became pregnant; the other did not. d) In 3 patients having normal secretory endometrium there were abnormally low concentrations of glycogen. *Two of them became pregnant;* the third did not.

COMMENT

There is a definite fluctuation in the glycogen content of the endometrium throughout the normal menstrual cycle. In general, the post-ovulatory levels are considerably higher than the pre-ovulatory ones. There is a marked peak at day 17, which is amply demonstrated and established by this work. We may speculate that this peak coincides with the

entrance of the blastocyst into the endometrial cavity, but this is merely an hypothesis. At any rate the peak does not seem to coincide with the time of rupture of the zona pellucida, which would be the ideal time to nourish the free blastocyst (14, 15). Furthermore, there is no indication that the presence of glycogen in the glandular epithelium during the early secretory phase means available glycogen for the blastocyst in the endometrial cavity, because the gland contents are not pure glycogen but some other compound.

When it is considered that the ovum can become implanted in the tube, on the ovary or on the peritoneal surfaces where the influence of the endometrium is not felt, we are probably justified in questioning the supposition that glycogen has anything to do with implantation. It seems just as reasonable to think of glycogen as a substance which is essential to the metabolism of the endometrium. Endometrium, in turn, is the precursor of decidua, which may be essentially a protective mechanism for the mother and not a convenience for the fetus.

Furthermore, when the results in our 18 infertility patients are analyzed, we are forced to question whether the level of glycogen concentration in the endometrium at the time of implantation has anything to do with successful nidation.

SUMMARY

1. Quantitative estimations of glycogen in endometrium have been carried out, using a modification of Petersen and Rose's anthrone method. A physiologic curve throughout the cycle has been established, based on the findings in carefully dated normal endometria, incorporating data from previous studies using the same method.

2. Glycogen concentration and distribution qualitatively have also been studied in a series of PAS-stained endometrial specimens, for comparison.

3. There was poor correlation between the qualitative PAS distributions and the quantitative anthrone values for glycogen. Staining methods should be supplanted by direct quantitative measurements.

4. A small number of abnormal endometria were processed for quantitative glycogen measurement and glycogen distribution and the results have been discussed.

5. Some concepts regarding the origin, metabolism and use of glycogen in endometrium have been questioned.

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9-α-FLUOROHYDROCORTISONE-INDUCED HYPERTENSION IN A MALE INFANT WITH ADRENOGENITALISM, AND IN 6 ADULTS WITH ADDISON'S DISEASE

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IN 1954, Fried and Sabo (1) reported that substitution of a fluorine atom in the 9-alpha position of the hydrocortisone molecule produced a marked potentiation of glucocorticoid effects; Borman *et al.* (2) reported a corresponding mineralocorticoid enhancement. This new compound, 9-α-fluorohydrocortisone, promised to become of major importance in antiphlogistic and maintenance adrenocortical therapy. In the course of time, it has become apparent that the potentiations observed are unequal; salt and water effects are enhanced more than anti-inflammatory effects, and consequently fluid retention as an undesirable side-effect is likely to occur at any dosage level producing a satisfactory antiphlogistic response. Thus Boland and Headley soon observed the occurrence of edema in patients with rheumatoid arthritis under treatment with 9-α-fluorohydrocortisone. This complication occurred in 12 out of 13 patients; in 6 of the 12 slight to moderate elevation of blood pressure also developed (3).

Though unsuitable for anti-inflammatory therapy, it seemed likely that the new compound might still prove valuable in the maintenance therapy of hypoadrenal patients. In an early report, Goldfien *et al.* indicated that 9-α-fluorohydrocortisone is satisfactory for this purpose but implied that, in order to avoid fluid retention, the dose must be kept so low that a small amount of cortisone is also necessary to restore a feeling of well-being. They also mentioned a patient with the adrenogenital syndrome in whom 2 mg. daily proved to be effective in suppressing corticotropin secretion without producing any side-effects (4).

Other studies of the clinical usefulness of 9-α-fluorohydrocortisone in the adrenogenital syndrome mention fluid and sodium retention as serious

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side-effects in 5 of 6 patients treated; there is no mention of any effect on the blood pressure (5).

It is the purpose of this report to present a case of the adrenogenital syndrome with adrenal insufficiency in a male infant, in whom marked hypertension without obvious fluid retention developed following therapy with fluorohydrocortisone. In addition, our experience with this drug in 6 cases of Addison's disease will be briefly summarized.

CASE HISTORY

J.W.H. (Duke-D-96874), an 8-week-old, white male infant was first admitted to Duke Hospital on September 20, 1954. The referral diagnosis was adrenal insufficiency. The mother's pregnancy and the gestation had been normal. Both parents were of limited intelligence and the patient's youngest brother was retarded in development; all other siblings were normal. The patient had weighed 7½ pounds at birth and had been breast-fed. There had been intermittent vomiting despite dietary manipulations. A gastro-intestinal x-ray series revealing gastric retention had led to exploratory laparotomy; a pyloromyotomy had been performed because of some pyloric hypertrophy. Following operation the infant had improved very little and had required prolonged administration of atropine. Because of some evidence of atropine toxicity, he was seen in consultation by one of us (T.B.W.), at which time the clinical picture suggested the diagnosis of adrenogenital syndrome. X-ray examination showed no advancement of bone age. Cortisone, in a dosage of 12.5 mg. daily, was begun and the patient was transferred to Duke Hospital for evaluation.

Upon physical examination, the temperature was normal and the systolic blood pressure was 50 mm. Hg. The child appeared dehydrated, malnourished and chronically ill, weighing 6¼ pounds. There was no clinical evidence of macrogenitosomia praecox.

Laboratory studies: the blood count was normal, and there were negative reactions to the tuberculin test and the serologic test for syphilis. Blood chemistry values were as follows: fasting sugar, 94 mg. per 100 ml.; NPN, 54 mg. per 100 ml.; serum sodium, 135 mEq. per liter; chloride, 77 mEq. per liter; and potassium, 5.8 mEq. per liter. Urinalysis revealed a persistent slight glycosuria which fermentation studies proved to be due to glucose. The excretion of 17-ketosteroids was 0.86 mg. per twenty-four hours. The patient was receiving no therapy except DCA at this time. X-ray examination of the chest and abdomen showed no abnormalities.

During an initial three-day period without cortisone, his clinical condition deteriorated, the serum sodium level falling to 103.9 mEq. per liter and potassium level rising to 7.2 mEq. per liter. Unfortunately, it was not possible to collect urine for steroid determinations at this time. In the light of the foregoing changes, the patient was treated for adrenal insufficiency with desoxycorticosterone acetate (DCA) and cortisone. He became asymptomatic and was discharged October 20, 1954, weighing 7⅞ pounds. He was receiving hydrocortisone in a dosage of 10 mg. orally twice daily, and was given 25 mg. of desoxycorticosterone trimethylacetate (DOC-TMA) intramuscularly at the time of discharge. Arrangements were made for him to return in a month for another injection and for re-evaluation.

Following discharge the patient did poorly for two weeks. Then an Addisonian crisis developed. He was admitted to a local hospital under the care of one of us (T.B.W.) and responded well to DCA and cortisone. After discharge he was followed closely and continued to thrive.

Several months later, it was our consensus that management might be facilitated if the patient could be maintained with a single medication. Therefore on March 8, 1955, DCA and cortisone were stopped and 9-α-fluorohydrocortisone was started in a dosage of 1 mg. orally per day. The transfer was uneventful, but after a month there was some increased frequency of stools and the child did not gain weight as readily as before; both of these conditions improved after increasing the dosage on April 19, 1955, to 1.5 mg. daily. Under this regimen, he successfully underwent two moderately severe respiratory infections without requiring extra salt or steroid therapy. By this time, reports in the literature and increased personal experience indicated that this dosage was likely to produce side-effects if long continued. On May 29, 1955, the patient was clinically asymptomatic but his blood pressure was 160/110 mm. Hg. Although the dosage of 9-α-fluorohydrocortisone was immediately reduced to 0.5 mg. daily, the blood pressure rose to 196/110 mm. Hg on June 2, 1955. Accordingly, he was again admitted to Duke Hospital on June 8, 1955.

On admission he weighed 17¼ pounds. The blood pressure was 300/240 mm. Hg and the pulse was 140. Pustular and acneiform lesions were visible over the forehead. In the ocular fundi there was an increased vasculature but no papilledema, hemorrhages, exudations, or arteriolar narrowing. The findings on physical examination were otherwise normal. There was no evidence of cardiomegaly, fluid retention, or excessive androgenic effects.

Blood counts were normal except for a leukocytosis of 18,850. Urinalysis findings were normal, except for minimal glycosuria on two of five tests. An eosinophil count was 377 per cu. mm. Blood electrolyte values were: serum sodium, 154.1 mEq. per liter; chloride 97.1 mEq. per liter; potassium, 3.1 mEq. per liter; and CO_2 combining power, 32.6 mEq. per liter. X-ray examination revealed that the heart was of normal size, and that the bone age was compatible with the chronologic age. An electrocardiogram was interpreted as indicating a normal heart save for a sino-auricular tachycardia of 136.

The patient's course in the hospital and his response to therapy are indicated in Figure 1. The blood pressure was checked several times daily and the values shown on the diagram are the most representative. Hydrocortisone in an oral dosage of 10 mg. twice daily was begun on June 8 and stopped on June 10. On June 13 he became cold and clammy, with a pulse of 170, although his blood pressure never fell below 120/100. At this time, the level of serum sodium was 139 mEq. per liter and of serum potassium, 7.1 mEq. per liter. The electrocardiogram showed spiked T waves, suggestive of hyperkaliemia. The administration of hydrocortisone, 25 mg. intravenously, and additional salt rapidly restored the child to normal. He was again given cortisone orally, 12.5 mg. twice daily, until June 20. At this time, therapy was again interrupted in order to investigate the pattern of steroid excretion. The gradual rise in urinary 17-ketosteroids, as shown in Figure 2, clearly confirmed the clinical impression of adrenal insufficiency associated with the adrenogenital syndrome.

This point having been established, the patient's maintenance therapy was finally adjusted at 1 mg. of DCA intramuscularly daily, and 37.5 mg. of oral cortisone daily. The urinary 17-ketosteroids had fallen to 0.6 mg. per twenty-four hours, when measured one week later. The blood pressure slowly and irregularly declined to a plateau of about 120/70 mm. Hg. The infant appeared clinically normal at the time of discharge on July 20, 1955, although his appetite was poor and for the last month in the hospital his weight had been stable at about 15¾ pounds. At no time did he demonstrate any conspicuous polydipsia, polyuria, or edema.

Since discharge, he has continued to do well. On October 20, 1955, his weight was

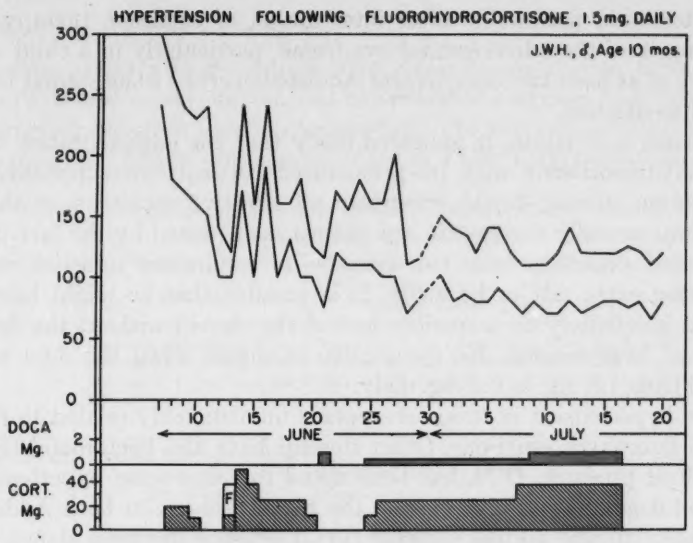

FIG. 1. *Infant J.W.H.* Improvement in hypertension after discontinuing 9-α-fluorohydrocortisone. Substitution therapy was interrupted June 10 in order to allow observation of the clinical response, and on June 21 for steroid excretion studies (shown in Fig. 2).

20 pounds 6 ounces and the blood pressure was 140/80 mm. Hg. The blood pressure reading may have been incorrect, in that it was obtained while the child was crying.

DISCUSSION

The infant with adrenogenitalism

This patient's clinical picture was typical of the male infant with the adrenogenital syndrome (6–8); the persistent vomiting and failure to thrive, the manifestations suggesting hypertrophic pyloric stenosis (to the point of prompting pyloromyotomy), and the dramatic improvement on cortisone were all entirely characteristic. The absence of any evidence of excessive androgen tissue-effects is not surprising, as corticotropin-suppressive therapy was instituted at such an early age. Finally, the progressive rise in

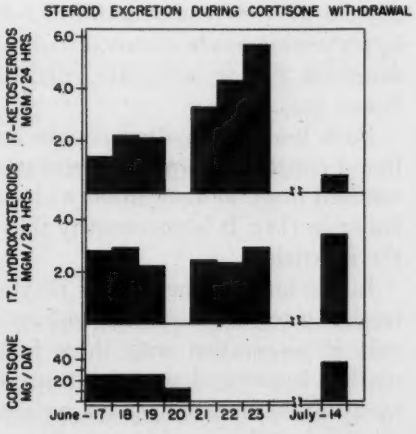

FIG. 2. *Infant J.W.H.* The rise in 17-ketosteroid excretion after omission of cortisone therapy is worthy of note.

17-ketosteroid production after interruption of cortisone therapy is the trademark of the adrenogenital syndrome, particularly in a child with a history of at least two unequivocal Addisonian crises when steroid therapy was discontinued.

In such a situation, it appeared likely that the administration of 9-α-fluorohydrocortisone with its pronounced corticotropin-suppressive and electrolyte effects, should constitute an effective maintenance therapy. This was actually the case in our patient, as attested by the fact that he recovered smoothly from two episodes of respiratory infection without requiring extra salt or hormone. It is possible that he might have continued indefinitely on a smaller dose of the steroid without the development of hypertension. No symptoms developed when the dose was reduced from 1.5 mg. to 0.5 mg. daily.

The hypertension in this case appears unmistakably related to the use of 9-α-fluorohydrocortisone. Other steroids have also been found to affect the blood pressure. DCA has been noted to cause some hypertension in normal dogs (9), and may elevate the blood pressure in both Addisonian patients (10) and normal subjects (11). Cortisone has been shown to elevate the tetraethylammonium floor in normotensive human subjects (12). In our patient the blood pressure gradually declined to normal during maintenance therapy with combined DCA and cortisone in dosages apparently just as adequate for maintenance as that of 9-α-fluorohydrocortisone.

Some patients with the adrenogenital syndrome exhibit spontaneous hypertension. This, too, appears to be due to the production of an abnormal steroid (compound S; 11-desoxy-17-hydroxycorticosterone). The hypertension has been noted to disappear along with the abnormal steroid excretion during adequate corticotropin-suppressive therapy with cortisone (13).

Both 9-α-fluorohydrocortisone and 9-α-fluorocorticosterone have been found capable of producing elevation of the blood pressure in adrenalectomized dogs, in association with polydipsia, polyuria, edema, and hypokaliemia (14). It is noteworthy that this effect can occur in the absence of the adrenals.

In the human, the reports previously cited (3–5) have emphasized the tendency toward fluid and sodium retention, the hypertension occurring only in association with these findings, if at all. Our patient exhibited marked hypertension without any evidence of fluid retention, unless the eventual stabilization of his weight at 1½ pounds less than that on admission be taken as evidence of loss of edema fluid. If so, edema was minimal, since the evidence for absence of both edema fluid and cardiomegaly was conclusive.

Six adults with Addison's disease

Our experiences with 9-α-fluorohydrocortisone substitution therapy in Addison's disease would suggest that hypertension and fluid retention may occur singly more often than in combination. The toxic effects observed in 5 patients with Addison's disease are listed in Table 1. Hypertension without edema developed in 2 men, and edema without hypertension developed in 3 women. (This apparent sex difference is probably fortuitous.) A sixth patient (not listed in Table 1) began therapy with 1.0 mg. daily. First, polyuria and edema developed; these manifestations disappeared without any change in dose. Several weeks later, while the patient was edema-free, progressive hypertension developed. When the dose was reduced at this time to 0.25 mg. daily, the blood pressure fell toward normal levels.

TABLE 1. TOXIC MANIFESTATIONS OF 9-α-FLUOROHYDROCORTISONE IN PATIENTS WITH ADDISON'S DISEASE*

Patient	Remarks	Fluorohydrocortisone dosage & effects	Present status
A.O., W ♂ 54 D-25012	Diagnosis 1946. History of hypertension 1934. Stabilized on 12.5 mg. E, 4 Gm. NaCl q.d. B.P. 130/80.	Begun Dec. 7, '54, 1.0 mg. q.d. B.P. rose slowly to 205/105 by Mar. 29, '55. Headache, retinal arteriolar constriction. No edema or weight gain.	While on E and DCA, B.P. fell to 140/80. At present on 0.25 mg. FF, & 25 mg. E. q.d. B.P. 168/90 on Nov. 1, '55.
M.J.P., W ♂ 48 B-94182	Diagnosis 1946. Stabilized on 10 mg. F b.i.d., 1.0 mg. DCA q. 2 d. B.P. 128/88.	Begun Jan. 1, '55, 1.0 mg. q.d. B.P. 168/90 on Apr. 1, '55. No edema.	On 20 mg. F and 0.25 mg. FF q.d. B.P. 150/92 on Aug. 19, '55.
H.S., W ♀ 36 D-38458	Diagnosis 1951. Stabilized on 25 mg. E q.d., and one 125-mg. DCA pellet. B.P. 110/70	Begun Mar. 15, '55, 5.0 mg. q.d. By Mar. 25, '55 had gained 10 lbs. Edema, headache; no hypertension. Continued edema when dose reduced to 2.5 mg. q.d.	Edema disappeared after FF discontinued. No hypertension on 25 mg. E q.d. and one 125-mg. DCA pellet.
V.L., W ♀ 47 C-68971	Diagnosis 1949. Stabilized on 25 mg. E q.d. and 25 mg. DOC-TMA q. 4 wks. B.P. 130/95.	Begun Mar. 15, '55, 1.0 mg. q.d. By Mar. 20, '55 had gained 12½ lbs. Generalized edema. B.P. 104/70 on Apr. 12, '55.	Changed to 0.25 mg. FF and 25 mg. E q.d. Gained 6 lbs. more. Dyspnea and joint pains. B.P. 112/80. Changed again to 25 mg. E q.d. and 25 mg. DOC-TMA q. 4 wks. No more edema.
I.K., W ♀ 51 D-8862	Diagnosis 1954. Stabilized on 25 mg. E q.d. and 25 mg. DOC-TMA q. 4 wks. B.P. 120/56.	Begun Apr. 5, '55, 1.0 mg. q.d. By Apr. 12, '55 had gained 10½ lbs. Dyspnea, substernal pain. Dose reduced to 0.25 mg. q. 2 d. Wt. still up 13 lbs. by Apr. 29, '55. B.P. 130/80 at that time.	Changed to 0.25 mg. FF q. 2 d. and 25 mg. E q.d. On Aug. 19, '55, no edema. B.P. 118/70

* Abbreviations: E = Cortisone
F = Hydrocortisone
FF = 9-α-Fluorohydrocortisone
DCA = Desoxycorticosterone acetate
DOC-TMA = Desoxycorticosterone trimethylacetate

Four of our patients are now being maintained on 9-α-fluorohydrocortisone (0.25 mg. daily), with either cortisone (12.5 mg. twice daily) or hydrocortisone (10 mg. twice daily) in addition. This regimen has proved satisfactory. Patient acceptance has been excellent and no untoward manifestations have occurred.

It appears that, even as substitution therapy, 9-α-fluorohydrocortisone is likely to have some toxic effects, the manifestations of which may vary widely between patients. In one patient arteriolar tone may be more affected; in another the renal tubules may be the primary target. In our experience, when edema occurs, it does so promptly; whereas when hypertension develops, it does so only after prolonged therapy over a period of weeks or months. If the initial dosage is excessively large, or if the patient is not followed carefully after beginning therapy, full-blown toxicity may develop, involving both hypertension and edema.

SUMMARY

Although 9-α-fluorohydrocortisone is effective oral maintenance therapy for patients with hypoadrenocorticism, the administration of 1.5 mg. daily to a 10-month-old male infant with the adrenogenital syndrome caused marked hypertension. This side-effect was not associated with any evidence of fluid retention. The hypertension gradually disappeared when therapy was changed to DCA and cortisone.

The hypertensive effects of adrenal steroid hormones appear to be related to their mineralocorticoid effects. In the present case, the lack of associated fluid retention indicates that this relationship is not always clear-cut. During treatment of 6 cases of Addison's disease with 9-α-fluorohydrocortisone the side-effects, in the main, were edema without hypertension early in the course of treatment, or hypertension without edema after prolonged treatment for weeks or months.

Acknowledgment

We are grateful to Dr. John R. Beem, Sharp & Dohme Laboratories, for the generous supplies of 9-α-fluorohydrocortisone which made this study possible

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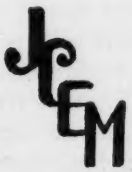

9-ALPHA-FLUOROHYDROCORTISONE ALONE AND COMBINED WITH HYDROCORTISONE IN THE MANAGEMENT OF CHRONIC ADRENAL INSUFFICIENCY

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SINCE the advent of cortisone in 1949, replacement therapy in chronic adrenal insufficiency has reached a more optimal level than had heretofore been possible. Cortisone acetate has been used alone and in combination with sodium chloride, as well as with various preparations of desoxycorticosterone. Thorn and his associates (1) have had extensive experience in the use of cortisone and of desoxycorticosterone in pellet form or as the trimethyl ester. For maintenance therapy they recommend cortisone in a dosage of 15 to 30 mg. daily together with desoxycorticosterone trimethyl acetate, 25 mg. every three weeks. We have been able to maintain our Addisonian patients on an average of 37.5 mg. of cortisone daily by mouth (given in three doses), with the addition of 4 to 6 grams of enteric-coated sodium chloride (2). The use of cortisone combined with desoxycorticosterone has been necessary only in 2 totally adrenalectomized patients in whom our usual replacement regimen did not correct the postural hypotension.

The use of combined cortisone and desoxycorticosterone replacement therapy has two possible advantages: 1) it lowers the daily requirement of cortisone, which is of some economic importance, and 2) it delays the onset of acute adrenal insufficiency should cortisone administration cease.

The synthesis of newer steroids with potent effects on electrolyte and water metabolism has led to an exploration of their use in chronic adrenal insufficiency, in an attempt to improve replacement therapy. This is becoming increasingly important, as the number of patients with chronic adrenal insufficiency is multiplying, particularly since the more widespread use of total adrenalectomy in the management of several disease states. We wish to report our experiences on the use of 9-alpha-fluorohydrocortisone alone and in combination with hydrocortisone in 8 patients whom we have observed for twelve to eighteen months. Kupperman and Epstein (3) recently recommended the administration of 10 mg. of hydro-

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cortisone twice daily, and 0.25 mg. of 9-alpha-fluorohydrocortisone every other day for optimal replacement therapy in chronic adrenal insufficiency.

CLINICAL MATERIAL AND METHODS

Metabolic balance studies were carried out in 3 patients with well established Addison's disease. Two of these received 9-alpha-fluorohydrocortisone alone and 1 received a mixture of 9-alpha-fluorohydrocortisone and hydrocortisone. The patients received a known protein, fat, carbohydrate and electrolyte intake and complete 24-hour urine specimens were collected. Nitrogen balance studies were conducted, employing a factor of 1.3 grams per twenty-four hours for the fecal nitrogen. Electrolytes were determined in serum and urine. The following technics were employed: sodium and potassium, by flame photometry using an internal lithium standard; urinary nitrogen, by the method of Peters and Van Slyke (4); urinary creatinine, by a modification of the Folin and Wu procedure (5); and ascorbic acid, by the method of Roe and Kuether (6).

After an initial control period, during which 2 of the patients were maintained on 50 mg. of cortisone acetate daily (administered in four equal doses orally) a dose of 2 mg. of 9-alpha-fluorohydrocortisone was given orally. The third patient had not had any replacement therapy for ten days before receiving one tablet of a mixture containing 0.1 mg. of 9-alpha-fluorohydrocortisone and 5 mg. of hydrocortisone every six hours.

The remainder of the study was carried out in the medical outpatient department of Royal Victoria Hospital on a group of 8 patients with chronic adrenal insufficiency. They were evaluated clinically at frequent intervals, at which times the levels of serum sodium, potassium and chloride were determined. These patients had all previously been maintained on cortisone acetate orally and supplementary sodium chloride.

RESULTS

The metabolic balance data on the 2 patients receiving 9-alpha-fluorohydrocortisone are charted in Figures 1 and 2. Both patients remained clinically as well as during maintenance therapy with cortisone acetate, but between the sixth and eighth days of 9-alpha-fluorohydrocortisone administration, edema became clinically apparent. This edema progressed in spite of a reduction of dosage from 2 mg. to 1 mg. daily.

In Patient E.C. (Fig. 1) there was a fall in urine volume with a concomitant weight gain of 5 Kg. during the sixteen days of observation. There was no alteration in nitrogen balance. A profound decrease in urinary sodium and chloride excretion occurred on the first day of 9-alpha-fluorohydrocortisone administration, with the development of a positive sodium and chloride balance. This was mirrored by the rise in the levels of serum sodium and chloride. There was no definite alteration in the potassium balance; in spite of this, the serum potassium level fell to 3.7 mEq. per liter. When the 9-alpha-fluorohydrocortisone dosage was reduced to 1 mg., there was a temporary increase in sodium and chloride excretion followed by a decrease.

Patient G.A. (Fig. 2) demonstrated somewhat similar findings. There was a weight gain of 6 Kg. during the eighteen days of observation. A

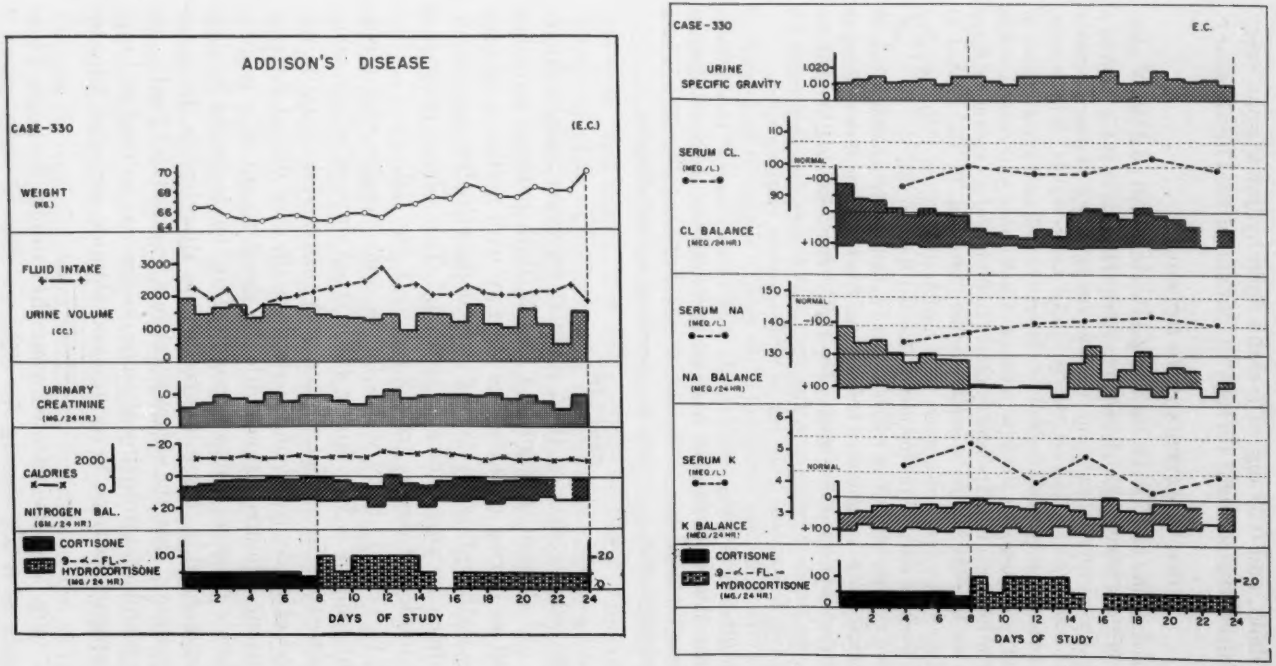


FIG. 1. Metabolic balance data during the administration of 9-alpha-fluorohydrocortisone to a 52-year-old man with Addison's disease.

There is a horizontal base line at zero. The intake is charted downward from this base line. The urinary and fecal excretions are then measured upward from the intake line towards the base line. If the output exceeds the intake, the final level will be above the base line; if the output is less than the intake, the final level will be below the base line. Thus a negative balance is indicated by a shaded area above the base line and a positive balance by a clear area below the base line.

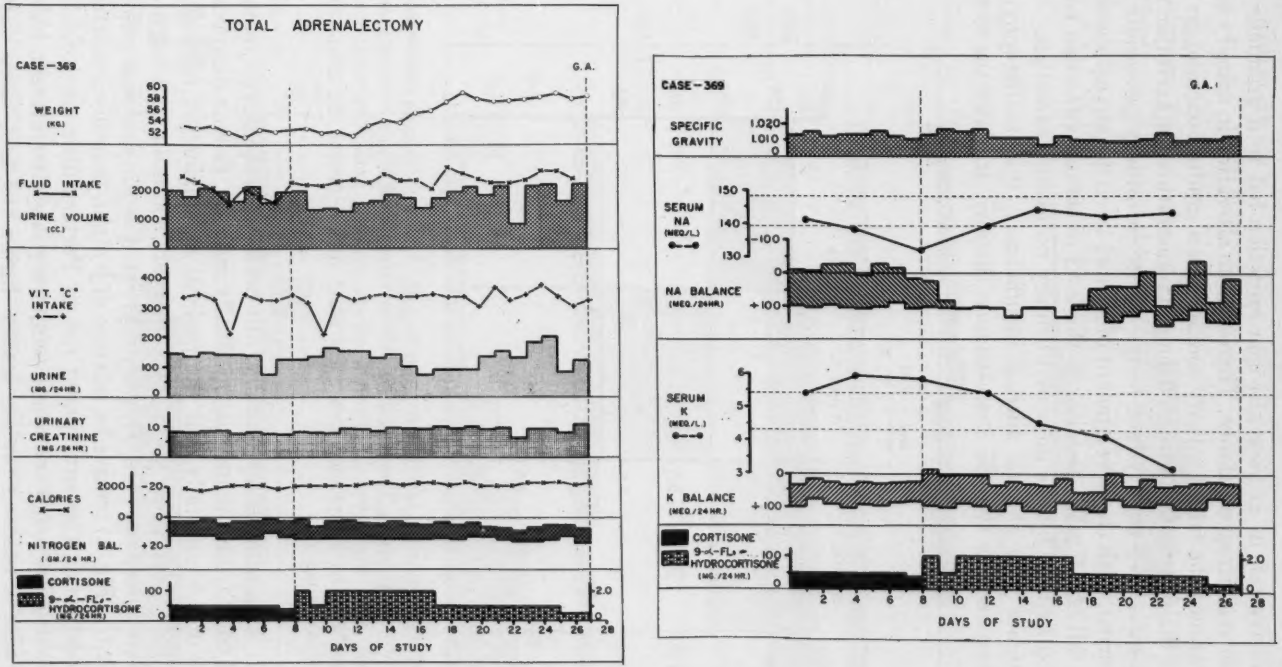


FIG. 2. Metabolic balance data during the administration of 9-alpha-fluorohydrocortisone to a totally adrenalectomized 28-year-old woman. (For key, *see* legend to Figure 1.).

slow progressive fall in urinary nitrogen excretion led to a gradually increasing positive nitrogen balance. There was no alteration in urinary vitamin-C excretion. The fall in urinary sodium was a gradual one and on the third day of 9-alpha-fluorohydrocortisone administration the excretion was less than 2 mEq. per twenty-four hours. A rise in urinary potassium excretion occurred, with the development of a short-lived negative potassium balance. A fall of 2.6 mEq. per liter in the level of serum potassium could not be accounted for by the increased urinary potassium excretion.

In 8 patients with chronic adrenal insufficiency, 9-alpha-fluorohydrocortisone was used as the sole maintenance therapy. Initially the dosage

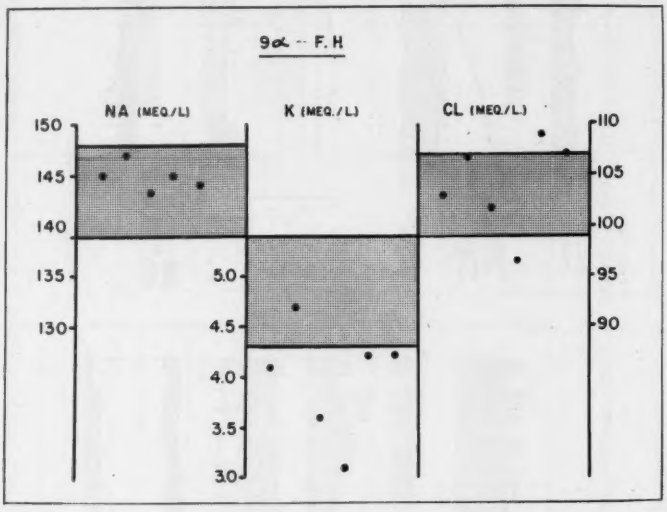
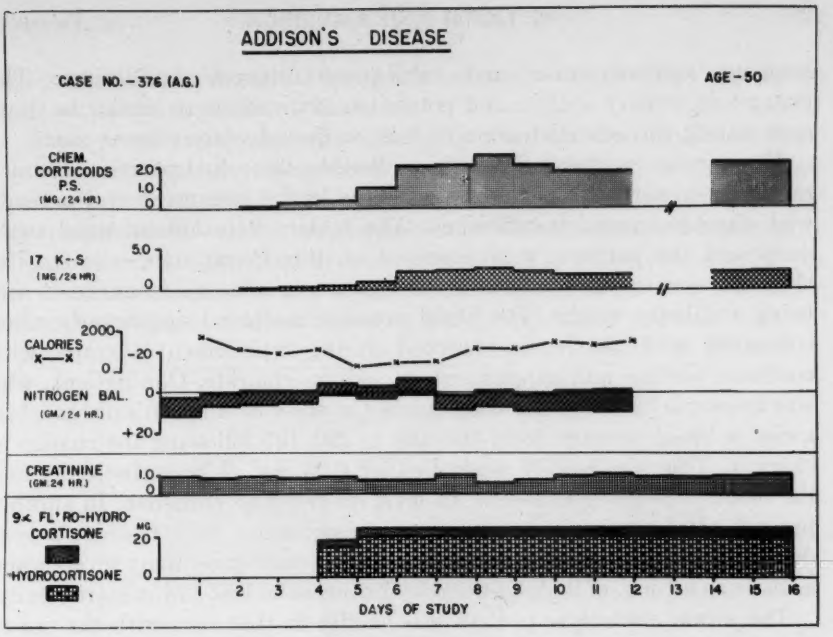


FIG. 3. Serum electrolyte levels in 8 patients with Addison's disease maintained on 9-alpha-fluorohydrocortisone. The serum potassium values were low, whereas the serum sodium values were high-normal. (Normal serum sodium, potassium and chloride values are indicated by the shaded areas. The black dots represent the means of the serum electrolyte values.)

was 1.5 mg. daily administered orally, but because of the development of generalized edema the amount was gradually reduced. Edema disappeared at a dosage of 0.5 mg. daily, but the patients complained of fatigue and weakness. There was a rise in the level of serum sodium and a fall in the level of serum potassium (Fig. 3), which persisted as the dose was reduced.

Patient A.G. (Fig. 4) received a mixture of 9-alpha-fluorohydrocortisone and hydrocortisone. The anticipated rise in Porter-Silber corticoids and 17-ketosteroids occurred. There was a temporary negative nitrogen balance associated with a reduced protein intake during the first three days of



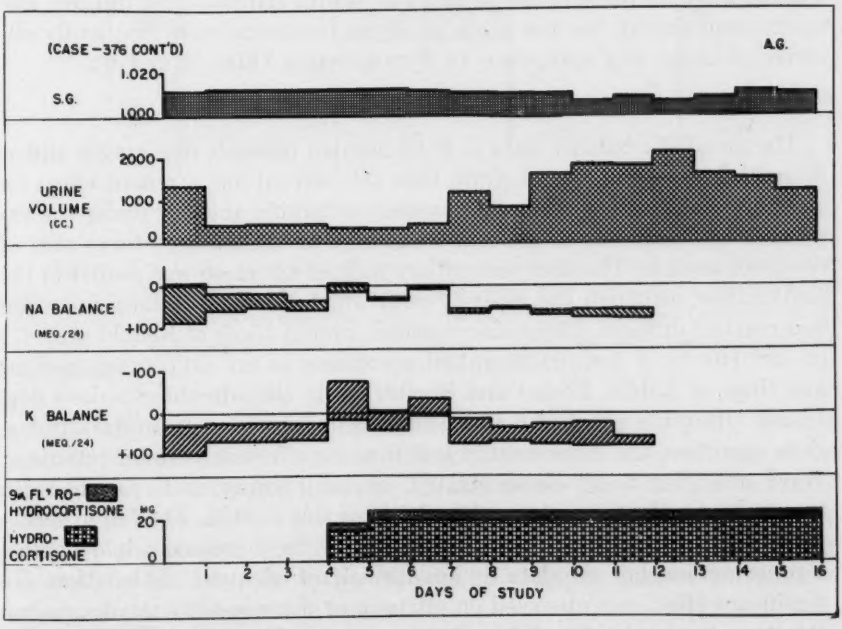


FIG. 4. Metabolic effects associated with the simultaneous administration of 9-alpha-fluorohydrocortisone and hydrocortisone, in a 50-year-old woman with Addison's disease. (For key, *see* legend to Figure 1.)

hormone administration, and subsequent nitrogen equilibrium. The changes in urinary sodium and potassium excretion were similar to those seen during the administration of 9-alpha-fluorohydrocortisone alone.

The mixture containing 0.1 mg. of 9-alpha-fluorohydrocortisone and 5 mg. of hydrocortisone per tablet was used in the treatment of 8 patients with chronic adrenal insufficiency. The tablets were administered twice daily and the patients were observed on this therapy for a year. The clinical response was excellent in all 8 patients, with maintenance of well being and body weight. The blood pressure increased significantly when compared with the levels observed during replacement therapy with cortisone acetate and supplementary sodium chloride. One patient, who was known to have hypertension before the onset of Addison's disease, had a rise in blood pressure from 160/100 to 220/130 following the change in therapy. This was readily controlled by 0.25 mg. of reserpine daily and the blood pressure returned to the level observed on cortisone. In another patient with previous hypertension in association with Cushing's syndrome, the blood pressure promptly rose to preadrenalectomy levels when as little as 0.1 mg. of 9-alpha-fluorohydrocortisone was administered daily.

The serum electrolyte pattern was similar to that seen with the use of 9-alpha-fluorohydrocortisone alone. The serum sodium level did not rise to the same extent, but low levels of serum potassium were frequently observed without any symptoms of hypokaliemia (Figs. 5 and 6).

DISCUSSION

The metabolic balance data in 2 Addisonian patients receiving 9-alpha-fluorohydrocortisone demonstrate that this steroid has a potent effect on electrolyte and water balance. Its sodium-retaining activity under the experimental conditions of this study was fifty to one hundred times that of cortisone acetate. The effect on urinary sodium excretion was similar in the two studies, although the rapidity with which maximal sodium retention was reached differed. These observations support those of Renold *et al.* (7) on the effects of 9-alpha-fluorohydrocortisone in an Addisonian patient and those of Liddle, Pechet and Bartter (8) in the adrenalectomized dog. In our study the effect of 9-alpha-fluorohydrocortisone on urinary potassium excretion was considerably less than its effect on sodium retention. Ward and Hench (9) demonstrated similar findings in a patient with rheumatoid arthritis receiving 4 to 8 mg. of this steroid. The fall in serum potassium level could not be accounted for by the increased urinary potassium excretion, but the data do not furnish an adequate explanation. No significant effect was observed on nitrogen or vitamin-C metabolism when the steroid was administered orally in a dosage of 1 to 2 mg. daily. Renold and his associates (7) demonstrated effects on intermediary metabolism

with a dose of 25 mg. administered intravenously over an eight-hour period in a patient with Addison's disease.

The increased mineralocorticoid activity of 9-alpha-fluorohydrocortisone and the prolonged duration of action when taken by mouth suggested its use in the treatment of Addison's disease. Small quantities administered by mouth would satisfy the requirements for salt-retaining hormone in these patients and it would become unnecessary to administer supplementary sodium chloride or desoxycorticosterone. Its use as the sole maintenance therapy in Addison's disease was disappointing and had to be dis-

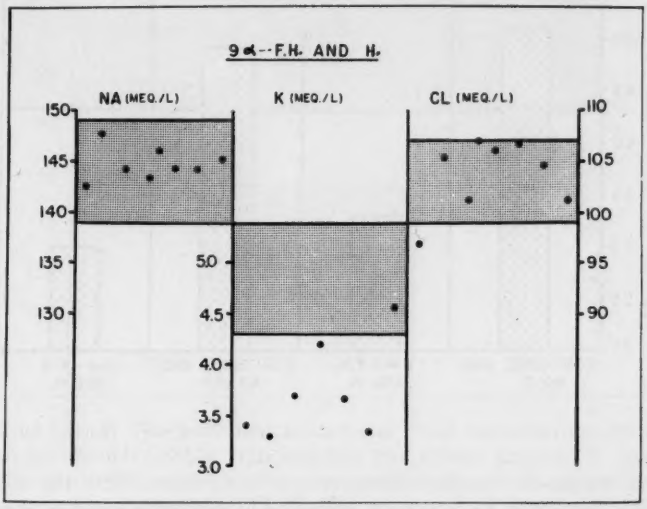


FIG. 5. Serum electrolyte levels in 8 patients with Addison's disease maintained on a mixture of 0.1 mg. of 9-alpha-fluorohydrocortisone and 5 mg. of hydrocortisone administered twice daily. The serum potassium levels were low and the serum sodium and chloride levels were normal. (Normal serum sodium, potassium and chloride values are indicated by the shaded area. The black dots represent the means of the serum electrolyte values.)

continued because of the undesirable side-effects, particularly massive edema. Excessive fluid retention did not occur when only 0.5 mg. of the steroid was administered daily, but at this dosage level the glucocorticoid effect was so small that the patients neither felt nor looked well. Renold *et al.* (7) also pointed out that in almost all cases this dose must be supplemented by cortisone or hydrocortisone in order to achieve a feeling of maximum well-being in the patient.

Some of the patients with chronic adrenal insufficiency maintained on cortisone acetate and sodium chloride for long periods of time had low

levels of serum sodium and elevated levels of serum potassium (Fig. 7) in spite of an apparently excellent clinical response. During 9-alpha-fluorohydrocortisone administration this serum electrolyte pattern was reversed, and a normal level of serum sodium with a low level of serum potassium was almost invariably seen. The hypokaliemia was not accompanied by any clinical symptoms or signs. Ward *et al.* (9, 10) describe a case in which

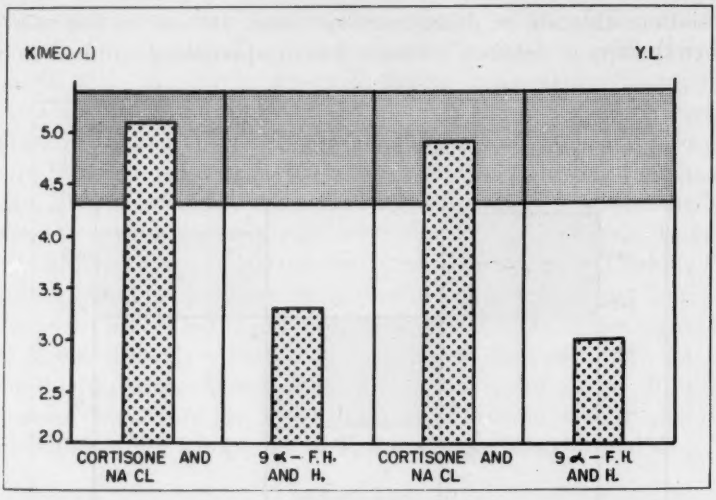


FIG. 6. Serum potassium levels in a patient with Addison's disease during the administration of cortisone acetate and supplementary sodium chloride followed by the mixture of 9-alpha-fluorohydrocortisone and hydrocortisone. Note the fall in serum potassium to low levels. No symptoms or signs of hypokaliemia could be elicited during these periods. (Normal serum potassium values are indicated by the shaded area. The patient's serum potassium levels are represented by the columns.)

the serum potassium level fell to 1.8 mEq. per liter without clinical evidence of hypokaliemia but with demonstrable electrocardiographic evidence.

Excellent results have been achieved with replacement therapy in chronic adrenal insufficiency, using the mixture of 9-alpha-fluorohydrocortisone (0.1 mg.) and hydrocortisone (5 mg.) given twice daily. The clinical state of these patients is not very different from that when receiving cortisone with supplementary sodium chloride, although all have higher blood pressure levels on the newer type of therapy. The changes in the serum electrolytes are similar in direction but less in degree compared to those seen with 9-alpha-fluorohydrocortisone administration alone.

It may be possible to arrive at more suitable proportions of the com-

bined steroids in the maintenance therapy of chronic adrenal insufficiency. Our data suggest that the amount of 9-alpha-fluorohydrocortisone might be reduced to 0.05 mg. per tablet or a total of 0.1 mg. per day. It might be even more physiologic to administer the 9-alpha-fluorohydrocortisone separately from the hydrocortisone or cortisone. This would allow complete individualization of replacement therapy. The sodium-retaining properties of 9-alpha-fluorohydrocortisone and aldosterone are comparable (11).

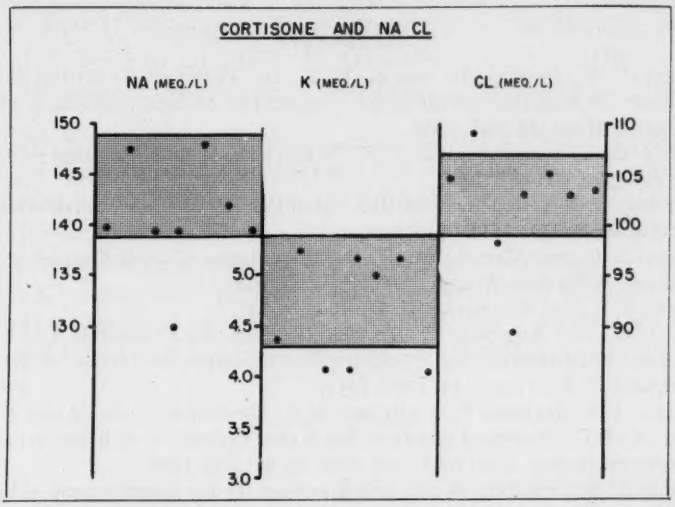


FIG. 7. Serum electrolyte levels in 8 patients with Addison's disease maintained on cortisone acetate and supplementary NaCl. The serum sodium levels were low-normal, whereas part of the serum potassium levels were at the upper limit of normal. (Normal serum sodium, potassium and chloride values are indicated by the shaded area. The black dots represent the means of the serum electrolyte values.)

Therefore it would seem reasonable to assume that, as a form of replacement therapy in Addison's disease, the combination of 9-alpha-fluorohydrocortisone with either cortisone or hydrocortisone, is as physiologic as possible.

SUMMARY

1. A study has been made of the possible use of 9-alpha-fluorohydrocortisone alone or combined with hydrocortisone as replacement therapy in chronic adrenal insufficiency.

2. 9-Alpha-fluorohydrocortisone alone had a marked effect on urinary sodium and potassium excretion, but in doses sufficient to exert a glucocorticoid effect, edema always occurred.

3. A mixture of 9-alpha-fluorohydrocortisone (0.1 mg.) and hydrocortisone (5 mg.) twice daily in tablet form maintained 8 patients with chronic adrenal insufficiency as well as did 37.5 mg. daily of cortisone acetate with supplementary sodium chloride.

4. The development of a low serum potassium level in some patients, although not accompanied by clinical evidence of hypokaliemia, suggests that the daily requirement of 9-alpha-fluorohydrocortisone may be less than 0.2 mg.

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SEXUAL PRECOCITY DUE TO INTERSTITIAL-CELL TUMOR OF THE TESTIS: REPORT OF 2 CASES

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SEXUAL maturation in the male occurs normally when the interstitial cells of the testis (Leydig cells) are first stimulated by anterior pituitary gonadotropins. This event occurs normally at any time between the ages of 10 and 14 years. Rarely, a functioning tumor of these interstitial cells may arise before the age of 10, causing precocious puberty. In his recent book on precocious puberty, Jolly (1) was able to find only 16 such cases in the literature, and reported 1 more. Since publication of this book, 1 additional case has been reported (2). We wish to add 2 cases to this series.

CASE HISTORIES

Case 1

James G., an 8½-year-old colored male, was seen at a boys' reformatory where he had been institutionalized because of his aggressive and antisocial behavior. No early history was available. He was a well developed, muscular boy, much larger than normal for his chronologic age (Fig. 1), 60¼ inches tall and weighing 120 pounds. He had a moderate growth of facial, axillary and pubic hair, an adult penis, an enlarged left testis measuring 2×4 cm., and a small prepuberal right testis measuring 1×2 cm. X-ray examination of his hand and wrist revealed a bone age of 17 years (Fig. 2). A roentgenogram of the skull showed a normal sella turcica. His 24-hour urinary excretion of 17-ketosteroids was 25.7 mg. and his creatinine excretion, 1352.0 mg.

A left orchiectomy was performed, and on sectioning the testis a well encapsulated pigmented adenoma was found (Fig. 3). Histologic section showed a solid adenoma of the Leydig cells (Fig. 4). The uninvolved area of the testis contained only atrophic seminiferous tubules.

One month postoperatively, the 24-hour urinary excretion of 17-ketosteroids was 6.1 mg., and of creatinine, 1442.0 mg. Six months postoperatively, there was no regression of genital development. The right testis had increased to the size of 3×1½ cm., and the

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FIG. 1. Case 1. James G., 8½ years old. Note the adult genitalia and unusually muscular build.

boy had maintained his virile appearance. The 24-hour urinary excretion of 17-ketosteroids was 4.5 mg., and of creatinine, 1292.0 mg. Twelve months postoperatively, his abnormal behavior had sufficiently improved so that he was paroled from the reformatory.

Case 2

Bruce B., a 6-year-old white boy, had experienced rapid growth for eight months, acne for two months, and gynecomastia and growth of pubic hair for one month. He had had frequent erections, but no abnormal sexual interests.

On physical examination, he was 48 inches tall, weighed 53 pounds, and had an unusually muscular build (Fig. 5). Small acneform papules were present on his nose and cheeks. There was scanty hair on the upper lip, scanty pubic hair, and no axillary hair. He had bilaterally enlarged breasts. His penis was 7 cm. long, his right testis measured 1×2 cm., and the left testis was enlarged to 2×3 cm. During examination of the gonads, he had an erection. No definite prostatic tissue could be felt on rectal examination.

A roentgenogram of the wrist revealed a bone age of 13, and a roentgenogram of the skull showed a normal sella turcica. Urinary 17-ketosteroid excretion levels were not determined.

Because of the unilateral testicular enlargement, the left gonad was removed. Grossly, the tumor mass in the testis measured 1.5×2 cm., and occupied the greatest part of the testis. The cut surface was brownish and slightly mottled. Histologic section showed masses of Leydig cells, with no evidence of malignancy, compressing the inactive seminiferous tubules (Fig. 6).

Eighteen months postoperatively, there was no regression of the penis; the pubic hair, which had been shaved off for the previous operation, had grown again. No further erections had been noted. His height was 51 inches, and weight 61 pounds, and the muscular development had decreased. The breasts had regressed, and the right testis had not changed in size.

DISCUSSION

A functioning Leydig-cell tumor of the testis arising before the age of 10 is a rare entity. With the 2 cases reported here, the total in the world literature now numbers 20 cases. The diagnosis of this condition depends on the presence of a precocious pseudo-puberty, associated with unilateral testicular enlargement. Since the puberal changes are limited to those pro-

FIG. 2. Roentgenogram of hands and wrist of Patient J. G., showing bone age of 17. All the bones have fused except the epiphyses of the radius and ulna, and further growth in these areas will be minimal.

FIG. 3. Photograph of the cut surfaces of the testis of Patient J. G., showing the deeply pigmented character of the tumor.

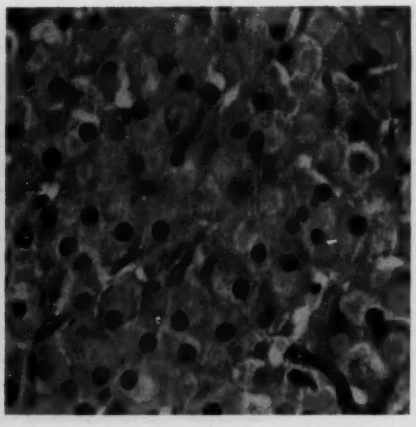

FIG. 4. Photomicrograph of section from the interstitial-cell tumor of the testis seen in Figure 3.

FIG. 5. Case 2. Physical appearance of Bruce B., aged 6 years. Note the enlarged genitals and the muscular build. His bone age was 13 years.

duced by androgen alone, this condition is differentiated from true precocious puberty, in that the spermatogenic function of the testis is dormant. Thus, the unaffected testis remains infantile. The enlargement in the involved testis consists of a small mass of atrophic tubules and a tumor composed of masses of interstitial cells. The condition must be differentiated from precocious pseudo-puberty secondary to an adrenal masculinizing tumor, in which condition both testes remain pre-puberal in size, and a tumor of the adrenals or an aberrant adrenal cell rest is found.

The determination of urinary 17-ketosteroids is helpful in establishing the diagnosis of interstitial-cell tumor of the testis, since 17-ketosteroid excretion is usually elevated. In the case in which this determination was made, a high value of 25.7 mg. per twenty-four hours was found (normal value for 8

FIG. 6. Photomicrograph of section from the interstitial-cell tumor of the testis in Case 2. Note the similarity to Figure 4.

years of age is 1.0 to 3.0 mg. per twenty-four hours). Following removal of the diseased testis, the 17-ketosteroids rapidly fell to normal.

This condition is treated by excision of the involved testis. In only 1 of the 20 reported cases was the tumor malignant (3). Following removal of the tumor, there is no significant regression of sexual hair or enlargement of the penis, but the excessive androgenic stimulation is quickly corrected, as indicated by the prompt fall of urinary 17-ketosteroids in Case 1, and the decreased incidence of erections.

The chief problems in this type of precocious puberty are the excessive growth and the strength of these children, the marked acceleration of bone maturation with eventual adult dwarfism, and the possible sexual disturbances. Each of the patients reported here showed remarkable growth and strength, and in one instance the boy's aggressive character and social immaturity were such that he became a delinquent and was sent to a reformatory. Within a year after removal of the tumor, he was rehabilitated. The rapid bone growth is dramatically illustrated in the bone-age roentgenogram of Case 1—a bone age of 17 years was found in an $8\frac{1}{2}$-year-old boy. Although he was very large for his age, little further growth was possible, since only a few epiphyses remained open. As an adult, his height will be stunted. Fortunately, sexual drive is rarely present in these children and therefore causes no difficulty.

SUMMARY

1. Two cases of precocious pseudo-puberty due to interstitial-cell tumor of the testis have been reported, bringing the total number of such cases reported in the world literature to 20.

2. Diagnosis of this condition depends on the concomitant finding of sexual precocity and unilateral testicular enlargement.

3. Treatment consists of excision of the involved testis.

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BLOOD CORTICOIDS: THEIR MEASUREMENT AND SIGNIFICANCE—A REVIEW

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THE adrenal cortex elaborates several steroids that are intimately related to many of the vital processes indigenous to human metabolism. These steroids have been classified by various authors into three major categories: 1) those affecting salt and water metabolism *e.g.*,—the physiologic adrenal product now known as aldosterone; 2) those affecting glucose metabolism and gluconeogenesis,—*e.g.*, the physiologic adrenal product known as hydrocortisone or compound F; and 3) those acting as androgens or anabolic hormones and excreted as alpha-ketosteroid (androsterone) and beta-ketosteroid (dehydroepiandrosterone). The metabolic build-up and break-down of these steroids has been the subject of much research, as has also their separation, measurement, and positive identification. The influence of pituitary adrenocorticotropin (ACTH) as a stimulating and maintaining factor in the adrenal metabolism of steroids, forms an important part of the background for this research. This review will attempt to encompass many of these factors as they act on the level of the blood corticoids, as measured both in health and disease.

BACKGROUND

Biosynthesis of adrenal steroids

A great deal of work has been carried out by various groups on the synthesis of adrenal steroids. Controversy has arisen as to whether cholesterol is the only precursor of these steroids or whether acetate may also play a role. Many have stated that acetate as a precursor has to act through cholesterol to form adrenal steroids, whereas others have thought that cholesterol is not an obligatory intermediary.

Hellman and associates (1) administered C^{14} or tritium-labelled cholesterol and acetate orally to human subjects. When cholesterol was given, it was detected in the plasma within one hour and maximum radioactivity of the free and esterified cholesterol was reached in two to three days; when tagged acetate was administered in the same manner, peak activity was noted in the plasma in eight hours. In 1951, Zaffaroni, Hechter and Pincus (2) reported on the production of tagged hydrocortisone and corticosterone from either C^{14}-labelled acetate or cholesterol by bovine adrenal glands perfused with corticotropin. Haines and associates (3, 4) in 1951 and 1952 demonstrated the formation of tagged corticosteroids from C^{14}

acetate in pig adrenal slices incubated with corticotropin. In 1952 Haynes, Savard and Dorfman (5) observed the same phenomenon, using bovine adrenal slices. Hechter *et. al.* (6) then demonstrated that the conversion of acetate to corticoid need not involve cholesterol as an intermediary. This was suggested because the specific activity of hydrocortisone or corticosterone synthesized by perfused bovine adrenal glands was higher than that of any cholesterol fraction which could be isolated either from the perfused tissue or the perfused blood.

Stone and Hechter (7) then performed an experiment in an attempt to find exactly where corticotropin stimulates synthesis of cortical steroids. They perfused cow adrenals with C^{14}-labelled cholesterol, progesterone and acetate, both in the presence and absence of corticotropin. In their study, corticotropin markedly increased the incorporation of C^{14} into corticoid only when cholesterol C^{14} was used as a precursor. The combined results strongly indicated that the major *in vitro* effect of corticotropin was concerned with the step involved in the conversion of cholesterol to progesterone. The studies with acetate-C^{14} demonstrated a pathway of corticoid biosynthesis which was not significantly influenced by corticotropin, and which did not involve the formation of cholesterol as an obligatory intermediary. According to the scheme of corticosteroid synthesis evolving from their perfusion studies, cholesterol is transformed to pregnenolone and the process is influenced by corticotropin stimulation. Pregnenolone, in turn, can be converted to progesterone, 17-hydroxyprogesterone, 17-hydroxy,11-desoxycorticosterone, and hydrocortisone, or it can proceed by way of progesterone, desoxycorticosterone and then to corticosterone.

A series of papers then appeared, describing the stimulatory effects of corticotropin on perfused bovine adrenal glands and the effects of adrenal irradiation on steroidogenesis (8–12). In 1952 Savard, Kolff and Corcoran (13) determined the corticosteroid pattern in dialysates obtained from dogs through an artificial kidney and peritoneal lavage. The corticoids were found both in a free, lipid-soluble state and in a water-soluble, lipid-insoluble state. The latter group was made lipid-soluble by glucuronidase hydrolysis and presumably represented the glucuronide form. When both fractions were examined by paper chromatography, the principal corticosteroid in both was hydrocortisone; corticosterone was detected only in the conjugated fraction. Several other steroids, which were alpha-ketols but did not have the alpha-beta unsaturated ketones, were found. These resembled tetrahydro-hydrocortisone and tetrahydrocortisone, in that they were $C_{21}O_4$ instead of $C_{21}O_5$ compounds. In addition, they found hydrocortisone and corticosterone in dog adrenal-vein blood in a ratio of 2 to 1.

In 1953 Bush and Sandberg (14) examined ethylacetate extracts of

plasma obtained from healthy males and females after the infusion of corticotropin. They showed that, with or without corticotropin, hydrocortisone was the major free adrenal corticosteroid in plasma. Corticosterone was rarely found in these extracts. The ratio of hydrocortisone to corticosterone was greater than 5, but when corticotropin was administered the ratio of hydrocortisone to corticosterone changed in some instances. In addition they tentatively identified cortisone in plasma. Also in 1953, Romanoff, Hudson and Pincus (15) examined adrenal-vein blood obtained during adrenalectomy in a patient with prostatic carcinoma. This patient was given corticotropin just prior to and during operation. The authors recovered 4.5 mg. of crystalline hydrocortisone and 0.4 mg. of crystalline corticosterone. These substances were identified by melting point, infrared analysis, sulphuric-acid chromogens and ultraviolet absorption. They found no cortisone, desoxycorticosterone, progesterone or 11-desoxycortisone. Tentative identification was made of tetrahydrohydrocortisone, tetrahydrocortisone, Δ^4-androstene-3,17-dione and 11-β-hydroxy-Δ^4-androstene-3,17-dione. In 1955 Hudson and Lombardo (16) analysed 6 human adrenal glands and found hydrocortisone to be the major steroid. Adrenal-vein blood studies on 3 patients showed ratios of hydrocortisone to corticosterone of 4.13, 2.93, and 10.6.

In summary therefore, compounds identified in human adrenal-vein blood definitely are hydrocortisone, corticosterone and the 17-ketosteroid, 11-β-hydroxy-Δ^4-androstene-3,17-dione. Compounds tentatively identified are tetrahydrocortisone, tetrahydro-hydrocortisone and the 17-ketosteroid, Δ^4-androstene-3,17-dione. Compounds sought for but not found are progesterone, and desoxycorticosterone. Compound S and compound E have been tentatively found in more recent studies.

In 1954 Hellman and his group (17) infused C^{14}-labelled hydrocortisone in amounts of 0.25 mg. to 100 mg. of hydrocortisone over a 30-minute period in patients with carcinoma. Of the dose administered, whether small or large, some 50 per cent was excreted in the urine at the end of six hours, 72 per cent in one day, and 80 per cent during three days. In one subject 9 per cent of the radioactivity was found in the feces during four days. In general, more than 90 per cent of the radioactivity administered was excreted in the urine and feces over three days. There was no evidence of an *in vivo* break-down of the steroid nucleus as determined by CO_2 analyses. Within twenty-four hours, 70 to 80 per cent of the radioactivity was excreted in the urine in conjugated steroids at a rate characterized by an average half-life of 3.6 hours. Apparently hydrocortisone was metabolized and conjugated rapidly by the liver, as the unaltered hormone accounted for only 10 per cent of the blood radioactivity within fifteen minutes after completion of the infusion. The rate of excretion and the

cumulative excretion were independent of the amount of hormone injected *i.e.*, whether the patient was saturated or unsaturated with regard to his need of the hormone.

Role of the liver

When adrenal steroids are administered exogenously or are produced endogenously they eventually get to the liver, where a large part of the steroid is conjugated into the glucuronide. This is then returned to the blood stream, where it circulates as both free and conjugated corticoid. Studies have shown that probably the largest part of circulating corticoid is in the conjugated form. The absolute level of circulating blood corticoids is the result of many factors acting at that particular instant. This level depends on the secretory state of the adrenal, the amount of corticotropin stimulating it, and hepatic and renal function. Tissue and protein binding and tissue utilization, metabolism and conjugation also play a part (18). Most of the steroid produced is eventually excreted in the urine. No steroid has ever been demonstrated to be metabolized to CO_2 and water. In 1953 Burton, Keutman, Waterhouse and Schuler (19) administered cortisone acetate intramuscularly to normal patients and to 2 patients with Addison's disease. In both groups hydrocortisone, tetrahydrohydrocortisone, tetrahydrocortisone and cortisone were isolated from the urine by paper chromatography. The authors postulated that cortisone is converted to hydrocortisone and then to tetrahydro-hydrocortisone, or that cortisone is converted directly to tetrahydrocortisone. At present, it is felt that tetrahydro-hydrocortisone and tetrahydrocortisone make up the largest part of the urinary corticoid excretion.

Miller and Axelrod (20) emphasized the importance of the liver in the metabolism of the steroids. They perfused cortisone contained in whole, heparinized homologous oxygenated blood through isolated, surviving normal and experimental cirrhotic rat livers. When extracted and chromatographed the following steroids were isolated from the normal livers: allopregnane-3β,11β,17α,21-tetrol-20-one, allopregnane-3α,11β,17α,21-tetrol-20-one, allopregnane-3β,17α,21-triol-11,20-dione, allopregnane-3α,17α, 21-triol-11,20-dione, and compound F. When cortisone was perfused through normal rat liver it disappeared rapidly. When it was perfused through cirrhotic rat liver it disappeared more slowly and also gave rise to two steroids not found in the normal liver. These were 6β-hydroxydesoxycorticosterone and allopregnane-17α, 21-diol-3, 11,20-trione.

METHODOLOGY

In 1943 Vogt (21) and in 1950 Paschkis (22) published papers pertaining to the bioassay of adrenocortical hormone. This represented the beginning

of such assays, which subsequently progressed to the more reliable chemical determination.

In 1948 Corcoran and Page (23) published one of the earliest methods for the chemical determination of corticosteroids in urine and plasma. They extracted heparinized whole blood with petroleum ether, after it had been precipitated with an alcohol-ether mixture. The dihydroxy-acetone side-chain of the aqueous phase was then oxidized to formaldehyde with periodic acid and the liberated formaldehyde equivalents were then measured by reaction with chromotropic acid. The peripheral blood levels that Corcoran and Page found in males and females ranged from 0.11 mg. to 0.42 mg. per 100 ml. Their values in the adrenal-vein blood of dogs ranged from 0.24 to 4.8 mg. per 100 ml. These values were much lower than those obtained with later methods.

In 1952 Mader and Buck (24) elaborated on a method of colorimetric determination of cortisone and related ketol steroids. Since the alpha-ketol grouping on the steroid molecule (as in cortisone, corticosterone or desoxycorticosterone) imparts reducing properties, it was felt that substances such as tetrazolium, which on reduction forms insoluble colored pigments known as formazans, would be useful in a colorimetric procedure. Alcoholic solutions of steroids that contain the primary alpha-ketol group reduce tetrazolium salts in the presence of tetramethylammonium hydroxide and form colored solutions. The authors stated that this method was sensitive to 10 gamma per ml. of cortisone acetate. Cortisone acetate, as well as desoxycorticosterone acetate, corticosterone, S acetate, and A acetate give rise to color with triphenyl tetrazolium and bisdiphenyl tetrazolium. In 1953 Chen, Wheeler and associates (25, 26) elaborated a method of analyzing corticosteroids, utilizing blue tetrazolium. They described two ways of performing this colorimetric procedure—one using methanolic sodium hydroxide and blue tetrazolium, and the other using ethanol, choline and blue tetrazolium. The values obtained were essentially in the same range as with methods involving measurement of formaldehydrogenic steroids, but the range of normal was much too narrow for routine use.

In 1952 Nelson and Samuels (27) published the first practical method adaptable to the routine determination of 17-hydroxycorticosteroids in blood. This method measured mainly hydrocortisone and depended on the presence of a 17,21-dihydroxy-20-ketone grouping. They extracted plasma, chromatographed the extract on magnesium silicate-celite or Florosil, and obtained three eluent fractions. The 17-hydroxycorticosteroids were in the third fraction, and were measured by the Porter-Silber color reaction. The authors stated that the color reaction followed Beer's law. Recoveries of 1 to 4 micrograms of hydrocortisone added to 10 ml.

of plasma and run through the Florosil column varied from 55 to 116 per cent. The compound in the third eluate was demonstrated to be hydrocortisone by paper chromatography. With this method, normal blood levels ranged from 4 to 10 micrograms per 100 ml.

Sweat (28) in 1954 elaborated on a procedure involving sulphuric acid-induced fluorescence of corticosteroids. Steroids were induced to fluoresce by means of concentrated sulphuric acid and this fluorescence was then measured on a Beckman spectrophotometer. Five steroids of the pregnane series produced intense fluorescence in ethanolic sulphuric-acid solution. These steroids were hydrocortisone, epihydrocortisone, corticosterone, epicorticosterone, and Δ^4-pregnane-11β,17α,20β-21-tetrol-3-one. From these studies the author made the following generalizations: 1) the presence of an hydroxyl grouping at carbon 11 seemed to be necessary for increased fluorescence, and 2) the presence of an alpha-beta unsaturated ketone in ring A also seemed to exert a minor influence on fluorescence. In 1955 Sweat (29) published a method for the measurement of adrenocorticosteroids in the peripheral and adrenal venous blood of man, which depended upon this colorimetric procedure. He extracted blood with chloroform, and partitioned and chromatographed the extract on silica-gel. The effluent fractions from the column were then quantitatively analyzed for corticosterone and hydrocortisone, using phenylhydrazine and also fluorescence. With this method, human adrenal-vein blood was found to contain relatively high concentrations of hydrocortisone and corticosterone. In a patient with Cushing's syndrome, the blood level of corticosterone was 203 micrograms per 100 ml., and of hydrocortisone, 880 micrograms per 100 ml. Human peripheral blood, using the foregoing method and fluorescence, apparently contained hydrocortisone and corticosterone. Additional paper chromatographic studies also revealed what seemed to be compounds S, A and E. The average concentration of hydrocortisone that Sweat found in normal peripheral blood was 10.8 micrograms per 100 ml., and of corticosterone-like compound, 4.3 micrograms per 100 ml. When corticotropin was administered to normal patients there was a two- to five-fold increase in F-like and B-like steroids in peripheral blood. When corticotropin was given to Addisonian patients, there was no significant increase in the level of plasma steroids as determined by fluorescence. Sweat also felt that tetrahydro-hydrocortisone rather than hydrocortisone is the substance that circulates in the blood as the glucuronide. The method appears reliable, but any procedure utilizing fluorescence for quantitative measurement requires expert technique and is not readily adaptable to routine use. Too many contaminants may interfere with accurate determination of the degree of fluorescence.

In 1953 Bondy and Altrock (30) published a method for the analysis of

17-hydroxycorticosteroids in plasma. It involved a complex extraction of plasma through several steps. The final extract was obtained in a chloroform-carbon tetrachloride mixture which was then evaporated to dryness and the Porter-Silber color reaction applied. From 60 to 70 per cent of hydrocortisone acetate was recovered when this steroid was added to plasma. There appear to be many steps in the extraction where steroid recovery could be diminished. Their normal mean value was 7.8 ± 3.3 micrograms per 100 ml. Hypopituitary patients had values of 2 micrograms per 100 ml.; Addisonian patients, 1.2 micrograms per 100 ml.; and adrenalectomized patients, 2.3 micrograms per 100 ml. When corticotropin was given, there was an increase in plasma levels. Studies on adrenal-vein blood revealed that the concentration of chromogen was higher than in peripheral blood. The rate of secretion of adrenal hormones was estimated by simultaneously collecting samples of left renal-vein and renal-artery blood and determining the difference in hormone concentration. This difference was multiplied by the value for renal plasma flow. The calculation revealed a secretion of 15 to 25 milligrams per day, which was stated to be approximately equal to the amount necessary to maintain patients with adrenal insufficiency.

In the same year Morris and Williams (31) published a method on the polarographic estimation of steroid hormones. These authors extracted heparinized blood with various solvents in a series of steps. The extracted corticosteroids were then separated by partition chromatography and, polarography was carried out on the separated fractions for quantitative measurement and identification. When compounds A, B, E, F and S were added to blood, recoveries were better than 80 per cent. In normal plasma they found the concentration of compound A to be 3.6 micrograms; compound B, 7.8 micrograms; compound E, 3.9 micrograms; and compound F, 8.4 micrograms, per 100 ml. In pregnancy, the values were elevated, mostly in the B and F fractions. The values in all fractions rose after administration of corticotropin; but the greatest rises were in the F fraction, as has been the finding of most other authors. Morris and Williams concluded that the 17-hydroxycorticosteroids occur mainly in the plasma rather than in whole blood. They also claimed to have found compounds A, B and E in blood, contrary to the findings of Nelson *et al.* (27) who found only hydrocortisone.

In 1953 Bayliss and Steinbeck (32) modified the method of Nelson and Samuels slightly. This involved a modification of the Porter-Silber (33) color reaction, namely, running column blanks which contained the solvent alone and also known amounts of hydrocortisone along with the unknowns. They made their corrections in this manner instead of applying Allen's corrective formula, as did Nelson and Samuels. When hydrocortisone was

added to plasma, recoveries ran from 68 to 93 per cent. The authors attempted to characterize the steroids found in peripheral blood by paper chromatography, and found that from a third to a half of the color developed was due to hydrocortisone, the remainder being due to more polar compounds. Once again, these findings differed from those of Nelson and Samuels.

In 1954 Silber and Porter (34) published a method for the determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma. Their method involved chloroform extraction of plasma to which hydrocortisone (5 micrograms) was added to allow for any loss of steroid in the extraction procedure; this amount was subtracted at the end of the analysis. An attempt was made to diminish the number of steps in the procedure so as to minimize loss of steroid. Thus the alcoholic phenylhydrazine-sulphuric acid reagent was added directly to the chloroform. The steroid was extracted from the chloroform into this reagent, and the chloroform was removed. They then applied their already well known colorimetric procedure. The average plasma level in human subjects was 13.3 micrograms per 100 ml. The method measures the 17,21-dihydroxy-20-keto configuration, with maximal absorption at 410 micra. Compounds B and A, and desoxycorticosterone reacted with the Porter-Silber reagent, but absorption peaks were at 340 to 360 micra. This peak seemed to be primarily due to the 3-phenylhydrazone. Color reaction also took place at room temperature, was complete in about eight hours, and remained stable for at least another twelve hours. The method is a good one and is readily adaptable to routine and research use, but it may not be quite as accurate as that of Nelson and Samuels.

Weichselbaum and Margraf (35) felt that most of the available methods for the determination of plasma corticosteroids measured only the 17-hydroxylated corticosteroids. They therefore combined the Porter-Silber procedure (which measures the 17-hydroxylated corticosteroids) with the Mader-Buck blue-tetrazolium procedure (which measures the 17-hydroxycorticosteroids as well as the 17-desoxycorticosteroids) and thus measured the 17-desoxycorticosteroid fraction by subtracting the amount of Porter-Silber chromogen from the amount of Mader-Buck chromogen. They extracted steroid from blood with ethyl acetate. After further refining, they divided their extract into two fractions. One fraction was chromatographed on Florosil for the Porter-Silber reaction and another fraction was chromatographed on silica (using formamide as the stationary phase) for the Mader-Buck blue-tetrazolium reduction reaction. Plasma was also hydrolyzed with bacterial glucuronidase in order to obtain the hydrolyzed glucuronides apart from the free form. The average value obtained for the plasma level of free 17-hydroxylated corticosteroid in

human subjects was 9 micrograms; of conjugated 17-hydroxylated corticosteroid, 17.6 micrograms; and of free 17-desoxycorticosteroid, 8.7 micrograms, per 100 ml. Their recoveries averaged 86 per cent for hydrocortisone by the Porter-Silber reaction, and 88 per cent for corticosterone by the Mader-Buck, Porter-Silber color reactions. This would appear to be a most exacting method.

Kassenaar and associates (36) extracted plasma with chloroform and put the extract through a series of refining steps. The residue was then subjected to a modified Porter-Silber color reaction. The normal plasma level ranged from 6.1 to 6.3 micrograms per 100 ml.—lower than with most methods. In Cushing's syndrome the plasma level was 20 micrograms per 100 ml., and after administration of corticotropin to normal subjects values were up to 38 micrograms per 100 ml.

Bongiovanni *et al.* (37, 38) incubated serum with beta glucuronidase and then dialyzed the serum. The dialysate was extracted with chloroform and the residue put through a Florosil column. The third fraction, obtained as in the method of Nelson and Samuels was then run through the Porter-Silber colorimetric procedure. After such hydrolysis, the values were much higher than those obtained without hydrolysis. The assumption was that a good part of the circulating blood corticoid is in the conjugated form.

In 1955 Klein and associates (39) extracted serum by the method of Nelson and Samuels for free corticoids. They then acid-hydrolyzed the extracted serum and obtained values for what they felt to be conjugated steroid. With this method, the plasma levels of free corticoids in adults and children averaged 13 micrograms per 100 ml. Their values for the levels of conjugated corticoids in children averaged 4 micrograms per 100 ml. and in adults, 8.5 micrograms per 100 ml. It is interesting that in this method the values for conjugated corticoids were considerably lower than those for free corticoids. As will be pointed out later, this was probably due to the acid hydrolysis. When corticotropin was administered there were rises in the levels of both free and conjugated fractions; these rises were thought to be comparable.

CLINICAL APPLICATIONS

Corticoid levels in various endocrine conditions

Utilizing the Nelson and Samuels method, with which normal plasma values are 4 to 10 micrograms per 100 ml., Nelson *et al.* (40) studied several patients with Addison's disease and could not detect any blood corticoid. When corticotropin was administered to normal patients there was an increase in the level of measurable blood corticoid to a peak of 20 micro-

grams per 100 ml. The peak was reached sixty minutes after corticotropin was given intravenously; values had returned to the base line four hours later. When corticotropin was given intramuscularly, a peak was obtained in two hours and values had returned to the base line six hours later. When corticotropin was given intravenously over a continuous period the increased levels were sustained and were higher than when corticotropin was given in any other way. Blood levels of steroids were found to be normal in acutely and chronically ill patients, but high in dying patients and those with terminal disease. It is likely that renal impairment contributed largely to the latter conditions. There were no measurable differences between arterial and venous levels of corticosteroids.

Bliss, working with this same group (41) found a *diurnal variation* in the blood corticoids, the highest values being present in the early morning. The level tended to drop to a lower plateau during the day. This finding has been substantiated by other investigators. Wallace, Christy and Jailer (42), utilizing the Silber-Porter method, found in normal subjects an average plasma level of 16.0 ± 6.9 micrograms with a range of 4 to 32 micrograms per 100 ml.—a fairly wide range. There was the usual overlap of values in the extremes of this range in patients with various adrenal disease. Day-to-day levels varied widely, as has been noted with other procedures. In hospital patients with renal disease there was generally a normal range of corticosteroids in blood, but in a few there were elevated levels. It is interesting that in this category of patients the urinary corticoids were low. Under these circumstances one would have expected increased plasma levels. In 9 pregnant patients studied in the third trimester, plasma levels of 22 to 52 micrograms per 100 ml. were obtained, some reaching the values observed in Cushing's syndrome. Patients with Addison's disease had levels of 0 to 21 micrograms per 100 ml. However, in 2 patients given corticotropin, there was no rise. This emphasizes further that Addisonian patients may have normal resting levels of blood corticoid, but there is no response to corticotropin. In hypopituitary patients with secondary adrenal insufficiency there was a range of 0 to 12 micrograms per 100 ml. In Cushing's syndrome the values ranged from 29 to 107 micrograms per 100 ml. After bilateral subtotal adrenalectomy was performed on these patients the values became normal. In congenital adrenal hyperplasia the values varied from 0 to 20 micrograms per 100 ml. Perkoff *et al.* (43), using the Nelson and Samuels method with a revised normal range of 13 ± 6 micrograms per 100 ml. (which approximates that of the Silber-Porter method), demonstrated that in diabetic coma the level of blood corticoids was elevated, and upon recovery the level fell again to within the normal range.

Corticotropin i.v. as a test of adrenal function

Bayliss and Steinbeck (44) employing their own method (normal values, 3 to 16 micrograms per 100 ml.), administered corticotropin intravenously to a series of patients and obtained a slight increase in the level of plasma corticoids within fifteen to thirty minutes, a definite increase in one hour, and a levelling-off for the remainder of the infusion. Values up to 54 micrograms per 100 ml. were obtained; when the infusion was stopped the level fell rapidly. This demonstrates the sensitivity of blood corticoid levels in reflecting adrenal function. When infusions of corticotropin were repeated daily over six to eight hours, a progressive rise in the plasma steroid level was obtained, suggesting that repeated daily stimulation increases the output of adrenal hormone. When a dose of 80 units of corticotropin in the form of ACTH-gel was given to patients in one injection, a significant rise of the plasma steroid level up to 36 micrograms per 100 ml. was observed in two hours, and a maximum concentration in four to eight hours. The level then usually fell off, with a return to control values in twelve to twenty-four hours after injection. Bliss *et al.* (45) administered 0.1 international unit of corticotropin in a single intravenous injection and noted a rise in the level of blood corticoids from 18 to 27 micrograms per 100 ml. in fifteen minutes. This is not a remarkable rise but demonstrates adrenal sensitivity to a very small amount of intravenous corticotropin. By four hours there was a fall to sub-control levels. Further studies by Eik-Nes *et al.* (46, 47) revealed that 25 international units of corticotropin given intravenously over six hours caused maximal stimulation of the adrenals and a maximal steroid response in plasma. In Addisonian patients there was a decreased response to this test, even when control levels were normal, as has been the finding of other investigators. On the basis of these observations they suggested the use of intravenous corticotropin as a test of adrenocortical capacity in the human. When they administered corticotropin to a patient with Cushing's syndrome due to adrenal adenoma, they obtained a hyperactive response and noted a change in plasma corticoids from a fasting level of 35 micrograms to a level of 100 plus micrograms per 100 ml. at the end of six hours. In various disease states such as hypertension, arthritis or diabetes, normal responses were obtained. In patients who had been maintained on cortisone for one to three months, there was a rise in the level of plasma corticoids after corticotropin—in thirty-six hours in one patient and in sixty hours in another.

Grumbach and associates (48) studied plasma corticoids in patients with Cushing's syndrome due to bilateral adrenal hyperplasia, using the Nelson and Samuels method. They found that many patients with Cushing's syndrome who had a normal concentration of free circulating corti-

coids had an increased concentration of conjugated circulating corticoids; the response to corticotropin was markedly hyperactive. Christy, Wallace and Jailer (49), employing the Silber-Porter method, found that the plasma level in normal patients rose from control values of 4 to 23 micrograms per 100 ml. to 35 to 54 micrograms per 100 ml. after intravenous administration of corticotropin for four hours. Patients with primary Addison's disease showed no rise after corticotropin, even if they had normal control values. In patients with hypopituitarism having control values of 0 to 12 micrograms per 100 ml., there were rises in the level of plasma corticoids after corticotropin, but in only a small percentage was the rise as high as in normal subjects. This response was observed after only one infusion; undoubtedly a greater response would have been obtained if intravenous corticotropin had been given over several days. Three patients with Cushing's syndrome due to adrenal hyperplasia showed rises from 34 to 77 micrograms per 100 ml., from 20 to 118 micrograms per 100 ml., and from 28 to 61 micrograms per 100 ml.—all of these representing a hyper-reactive response. After bilateral subtotal adrenalectomy, corticotropin elicited a subnormal rise in these patients. In congenital adrenal hyperplasia there were three types of response to corticotropin: 1) a minimal rise; 2) no rise, or 3) a normal rise. These results are hardly specific, but other investigators have reported that most patients with the adrenogenital syndrome exhibit responses 1 and 2. In patients with a normal pregnancy (50) these authors found that in the third trimester control values were 32 to 46 micrograms per 100 ml.; after corticotropin the values were 79 to 111 micrograms per cent which denotes a hyper-reactive response. Such hyper-reactive responses are not measurable with the Nelson and Samuels method, which involves Florosil chromatography. When corticotropin was given to patients with arthritis who were being maintained on prednisone, there was partial to complete prevention of the rise in the level of plasma corticoids. When corticotropin was given 1) to patients with Cushing's syndrome due to adrenal carcinoma, and 2) to a pregnant woman with Addison's disease, no rise in the level of plasma corticoids was obtained.

Bayliss, Broadbent and Steinbeck (51) infused a total of 20 units of Armour ACTH over an eight-hour period into a 41-year-old patient with the adrenogenital syndrome. There was a rise in the level of 17-ketosteroids but no appreciable rise in plasma corticoids from a control level of 3 micrograms per 100 ml. They gave 100 mg. of tetrahydrocortisone to 1 patient and observed no increase in plasma corticoid concentration. Since their method measured only the free form, they felt that tetrahydrocortisone entered the portal system and was conjugated by the liver. Eik-Nes *et al.* (52) administered hydrocortisone and cortisone intravenously to normal patients and found that the rate of disappearance of

these compounds from the plasma was related directly to their concentration in the plasma. A peak was obtained in one hour, with a return to control levels in six hours. The clearance of orally administered steroids from the plasma was variable; it was quicker following administration of the microsuspension than following tablets. A peak was obtained in one hour, with a return to control levels in eight hours. When the material was given intramuscularly, a peak was obtained in two hours and control levels had not been reached at the end of twelve hours.

Studies on the effects of such substances as epinephrine, insulin or histamine on the plasma corticoid level are mentioned here only for their bibliographic reference value (53–57).

Surgical states

Further studies on the changes in plasma corticoids have involved their determinations in surgical states as well as in liver disease. Franksson and Gemzell (58) conducted such studies in the preoperative period. They found a rise in the blood corticoid level from an average control value of 5.5 micrograms per 100 ml. two days before surgery, to 14.5 micrograms per 100 ml. on the day of surgery. They felt that this rise was due to psychogenic stress. Sandberg *et al.* (59) in a similar study on the effects of surgery found that the plasma corticosteroid concentration was elevated following the induction of anesthesia, and during and immediately after operation. The elevation following surgery was roughly proportional to the duration and severity of the operative procedure, and it usually persisted for at least six hours. They also observed the effects of infused corticotropin in these same patients, both preoperatively and postoperatively. Even when the postoperative levels of 17-hydroxycorticosteroids rose spontaneously to levels as high as those induced in normal subjects by corticotropin, the infusion of corticotropin postoperatively under these conditions led to an additional increment, indicating that the adrenals had not been maximally stimulated. When the metabolism of intravenous hydrocortisone preoperatively was compared with the metabolism postoperatively, it was noted that the patients did not metabolize the infused hydrocortisone as rapidly after operation as they did before. The authors concluded that an impaired metabolism of the circulating steroid also contributed to the rises observed. Since this was likely due to hepatic impairment, Tyler, working with the same group of authors (60), continued the study of the impaired metabolism of plasma corticosteroids by carrying out concomitant bromsulfalein (BSP) excretion studies. It was shown that the postoperative rises in the level of plasma corticosteroids were directly correlated with impaired BSP excretion. The conjugated urinary 17-hydroxycorticosteroids rose after all except one brief minor

procedure, but the rises were not correlated with the plasma levels attained. Their conclusions were that the increased plasma level of 17-hydroxycorticosteroids after surgery was the result of both increased adrenal secretion of these steroids and impaired hepatic removal.

Liver disease

Klein *et al.* (61) in 1955 measured both free and conjugated plasma corticosteroids by the method of Nelson and Samuels. The conjugated fraction was hydrolyzed by acid hydrolysis of the residue remaining after free corticoid had been extracted. With this method they noted that there was recovery of only 50 per cent of added pure hydrocortisone, but recovery of 90 per cent of tetrahydrocortisone. This study was specifically gauged to determine any difference between the level of serum corticoids in normal subjects and that in patients with liver disease. With their method the average normal serum (control) free corticoid level was 13 micrograms, and the average normal (control) conjugated corticoid level was 8.5 micrograms per 100 ml. In adult patients with cirrhosis, the average serum free corticoid level was 10 micrograms, and the conjugated corticoid level was 1.7 micrograms per 100 ml. The authors then administered cortisone acetate orally to normal subjects and noted a rise in the conjugated serum corticoid level which followed the rise in the free corticoid level. The elevated concentration of the conjugated corticoids persisted longer, and the peak occurred later than that of the free corticoids. However, two to three hours after cortisone acetate had been administered to cirrhotic patients, the free corticoid level roughly followed that found in the normal subject, except that the peak persisted about a hour longer. The conjugated fractions were initially at low levels and the rise was only minimal. When corticotropin was administered to normal subjects, there was a prompt rise in both the free and conjugated corticoid levels. The same amount of corticotropin given to patients with liver disease induced a normal rise in the free corticoid level but none, or only a slight rise, in the level of the conjugated fraction. The authors concluded that patients with advanced liver disease conjugate exogenous or endogenous corticoids slowly and that the rate of removal is slower than in normal subjects. In view of their data, the conclusions drawn are valid. However, in any method in which the procedure of hydrolysis results in such marked destruction of added steroid, it is readily understandable why these authors noted smaller amounts of conjugated corticoid than of free corticoid. This finding is contrary to the experience of other investigators. Brown *et al.* (62) infused hydrocortisone and tetrahydrocortisone in a series of normal subjects and in another series of patients with liver disease. When hydrocortisone was infused, there was an initial

rapid disappearance of the 17-hydroxycorticosteroids from the plasma in both normal subjects and patients with liver disease. One hour after the start of the injection only about 5 per cent of the infused steroid remained. Then came a period lasting many hours, during which the plasma 17-hydroxycorticosteroids disappeared at a slower rate. The rate was noted to be inversely proportional to the degree of liver damage, as measured by BSP excretion. This is in agreement with the postoperative findings previously mentioned. When tetrahydrocortisone was infused, it disappeared at a much more rapid rate, independently of liver function. The authors observed that patients with liver disease had normal fasting levels of plasma 17-hydroxycorticosteroids but excreted less urinary 17-hydroxycorticosteroid than did the normal subjects.

Pregnancy

Plasma corticosteroid studies in pregnant women, in normal children, and in children with various diseases have been carried out by many investigators in recent months. Bayliss *et al.* (63) recently reported studies on a series of pregnant patients. They measured the plasma corticoid level at monthly intervals in 30 pregnant women and noted that, after approximately the third month of gestation, there was a progressive rise that continued to term. After parturition the level fell to normal within one week. These authors also found an elevated level of corticoids in umbilical blood. Robinson *et al.* (64), in a similar study, found that the plasma corticosteroid level rose in pregnancy to a mean of about 33 micrograms per 100 ml. They stated that the level was of the same order of magnitude in both early and late stages of pregnancy. In contradistinction to the findings of Bayliss, these authors found that it generally took about six to nine weeks for the plasma corticosteroids to return to the normal postpartum level, though in some patients this did occur within a few days. Apparently this finding is unique with the Robinson group, because Gemzell and others have reported a return to normal levels within six days. In view of the known rate of disappearance of administered corticoid, and if the placenta be the major source of the increased plasma corticoid in pregnancy, one would expect a fairly rapid return to control levels (one week) after parturition. In an interesting study, Gemzell (65) found a maximum level of 20 micrograms per 100 ml. (not elevated) in pregnant women in the last trimester. However, in cases of cesarian section, although the mother's plasma corticosteroid level was essentially about 20 micrograms per 100 ml., the infant's plasma level was only 2.9 micrograms per 100 ml. The authors then studied the plasma corticosteroids in primiparous patients as well as in their infants, and found a mean level of 42 micrograms per 100 ml. for the mother, and 28

micrograms per 100 ml. for the infant. In a similar study on multiparous patients, the plasma corticosteroid level was 23.4 micrograms for the mother and 15.8 micrograms for the infant.

Newborn

Klein *et al.* (66) measured plasma corticosteroids in a series of 14 newborn infants, 2 to 5 days of age. The blood corticoid level was only 0.9 microgram per 100 ml. Normal infants ranging in age from 3 weeks on, had levels of 13 micrograms per 100 ml., as in the adult. The authors' hypothesis was that the maternal hormonal levels apparently depress the fetal pituitary and the low levels in the infant in the first week of life are due to depressed pituitary function. Then the infant's pituitary-adrenal axis begins to function normally and the blood corticoid level rises to its expected value. Carrying this one step further, the authors (67) in another study administered corticotropin to children and to 3-day-old infants. In the infants, the control values for plasma corticoids averaged 2 micrograms per 100 ml. One hour after infusion of corticotropin, the level rose to 26 micrograms, and two hours after infusion, to 45 micrograms per 100 ml. In normal children, the mean control level was 14 micrograms; one hour after corticotropin it had risen to 33 micrograms, and two hours after infusion, to 31 micrograms per 100 ml. Control levels in one infant averaged 0.5 microgram; this infant was then subjected to operative trauma and postoperatively the plasma corticoid level rose to 100 micrograms per 100 ml. The authors concluded that though the low level of plasma corticosteroid in the newborn suggests adrenal hypofunction, probably due to suppression of the fetal pituitary by maternal hormone levels, such hypofunctioning adrenals respond readily to stress and to stimulation by corticotropin. This would appear to be a reasonable and valid conclusion.

Children

Ely, Kelley and Raile (68–71) measured the concentration of plasma 17-hydroxycorticosteroids in children. They found that there was no significant difference in levels between children with various diseases and normal children. The only patients in whom low levels of plasma 17-hydroxycorticosteroid were found were those with the *adrenogenital syndrome.* In normal children the administration of corticotropin caused a prompt but transient elevation of the blood corticoid level. These authors then measured blood levels of 17-hydroxycorticosteroids in children with *acute rheumatic fever.* When the acute rheumatic fever had lasted for two weeks or more the mean blood corticoid level was 5.9 micrograms per 100 ml. Their mean value for normal children was 12 micrograms per 100 ml.

Low levels were also found in inactive rheumatic fever. The concentration of blood corticotropin was elevated in these groups. When exogenous corticotropin was administered, there was a normal rise in the level of blood corticoids. During the first week of acute rheumatic fever, blood levels of 23.1 micrograms per 100 ml. were observed, as well as low levels of corticotropin. The conclusion was that patients with acute rheumatic fever have a relative adrenal insufficiency but the adrenals can still respond to corticotropin. This conclusion has neither been generally acceptable, nor reproducible in other laboratories.

Comment

It is apparent that much progress has been made in the past few years relative to the evaluation of adrenal function. The availability of methods to measure the physiologic products of adrenal metabolism directly in the blood has served as a great stimulus to our understanding of the adrenal in health and disease.

SUMMARY

A review of the current literature pertaining to the status of blood corticoid determinations in the appraisal of adrenal function has been presented. Background studies, methodology and clinical applications of this useful clinical diagnostic and research procedure have been discussed.

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Letters to the Editor

THE QUANTITATIVE DETERMINATION OF PROGESTERONE IN HUMAN PLASMA

To the Editor:

The evolution of methods for the quantitative determination of the ovarian hormones and their metabolites in blood and in various body fluids and tissues, should lead to a better understanding of the role of these substances in physiology and pathology. In particular, the application of such methods might be expected to clarify the significance of changes in the levels of endogenous secretion and of such defects as may occur in the intermediary metabolism.

During the past four years, a research project has been undertaken in this laboratory with, as its objective, the development of a sensitive method for the chemical determination of progesterone and its metabolites in human blood. The task has been made more difficult by the fact that, for the application which was envisaged, it was essential to carry out repeated assays upon samples of plasma of the order of 5.0 to 10.0 ml. This report summarizes the progress which has been made in the determination of plasma progesterone. The determination of pregnanediol in plasma has been reported following intravenous administration of the hormone (1), but this metabolite has not yet been isolated from blood. Subsequent work upon this aspect of the method will be presented separately.

It was not until 1954 that progesterone was isolated from peripheral human blood (2, 3). Isolation was achieved by the application of paper chromatography to purified extracts of plasma and identification of the eluted hormone by infrared spectroscopy and other procedures. This technique has since yielded valuable information concerning concentration of progesterone in blood and tissues and has the important virtue that compounds other than progesterone may be detected. On the other hand it is not suitable for the comparative analysis of a series of single blood samples taken during the menstrual cycle or in pregnancy, and the values reported for pregnancy plasma (3) refer to pooled samples or extracts.

The starting point of the present investigation was a chromatographic technique which had been used for the determination of relatively large amounts of progesterone (4) and which, in common with other procedures (5), had failed to detect progesterone in pregnancy blood. Many recovery

experiments were carried out, in which different methods of extraction, purification, chromatography and spectrophotometry were extensively investigated. In these experiments, amounts of progesterone ranging from 1.0 μg. to 10.0 μg. were added to 10 ml. of plasma from male subjects; recoveries were checked with pure solution and other controls. It was necessary to devise a method of purification which would involve minimal loss of steroid, but which would still be adequate in eliminating interfering substances from the final product. A balance between extraction loss and spectrophotometric difficulties was finally achieved by extracting diluted, alkaline plasma with large volumes of an ether:methylene chloride mixture, followed by chromatography on a column of carefully standardized alumina and the formation of the iso-nicotinic acid hydrazone. Pretreatment with alkali is essential and the extract is clear and colorless. The plasma is diluted with an equal volume of N/3 sodium hydroxide, shaken mechanically for five minutes and then extracted three times with 15 volumes of the solvent mixture (*i.e.*, 5 ml. of plasma and 5 ml. of alkali are extracted with a total of 450 ml. of solvent). The processes of formation of the dinitrophenylhydrazone, the thiosemicarbazone and the iso-nicotinic acid hydrazone were investigated and the formation of the latter proved most successful. This hydrazone, which has a maximal absorption at 380 mμ had been used for the estimation of larger amounts of Δ^4-3-ketones (6). It was adapted to a micro-scale, using only 0.6 ml. of reagent and reading in a micro-curvette. The concentration of progesterone is calculated from a calibration curve based upon the Allen correction factors obtained from the pure steroid. Using this technique, the extraction loss is virtually eliminated, as demonstrated by the fact that recoveries from male plasma are similar to recoveries obtained when the hormone is added directly to the chromatographic column. In either series, they ranged from 80 to 95 per cent. The spectrophotometric curves derived from plasma or pure-solution recovery experiments resemble those derived from the pure steroid.

The final method has not yet been applied extensively, but it is encouraging to find that the values obtained for single blood samples taken during pregnancy are in agreement with the values obtained for pooled plasma (3). This also applies to values for placental venous blood. Thus levels obtained in pregnancy blood were: 0.098 μg., 0.110 μg. and 0.123 μg. per ml.; and in placental venous blood, 0.425 μg. and 0.500 μg. per ml. The high plasma concentrations observed immediately after intravenous infusion of the hormone (*e.g.*, 3.76 μg. per ml. after 150 mg. of progesterone) fell rapidly in the hour following the administration.

Although there was indirect evidence for the identity of the iso-nicotinic acid hydrazone, it was important to obtain more definite information on

this point. The final product of the new technique has recently been investigated by Dr. Zander and his colleagues at the Universitäts-Frauenklinik at Köln. They report that the appropriate fractions from the alumina chromatograms contain a substance in a highly pure state, with absorption maximum at 240 mμ and the same R_f value as progesterone. For these analyses to be carried out upon a single sample by the method of Zander and Simmer (7), it was necessary to raise the progesterone level of the blood by intravenous administration of the hormone.

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ANTIDIURETIC HORMONE (ADH) IN THE HUMAN FEMALE

TO THE EDITOR:

A method for the extraction of oxytocin and antidiuretic hormone (ADH or vasopressin) from rat's blood has been described by Bisset and Walker (1). We have slightly modified this method and used it to extract ADH from blood collected from: 1) non-pregnant women, 2) pregnant women in the last trimester, 3) women during labor, and 4) women in the first week after parturition, before and during suckling. These extracts were then assayed for ADH.

Materials and method. Venous blood from the antecubital fossa was taken from women in the foregoing categories. The individual blood samples were added immediately to acid-alcohol and extracted according to a modification of the method of Bisset and Walker (1). Antidiuretic activity of the extracts was determined by a method described previously (Robinson and Macfarlane (2)), in which intravenous injections are made into an assay rat receiving a constant water load and under ethanol anesthesia. The antidiuretic activity of the extracts was compared with that of known amounts of vasopressin (Pitressin; Parke, Davis & Co.) injected into the same rat, using a four-point assay. The method is sensitive to the injection of 10 μU of vasopressin and can discriminate 5μU over a range of dosages from 10μU to 80μU of vasopressin. The concentration of 5-hydroxytryptamine in the blood samples was far below its minimal antidiuretic dose (3).

Results and discussion. Table 1 shows the antidiuretic activity of the blood extracts investigated. This activity is almost certainly due to ADH because: 1) the activity is destroyed by sodium thioglycollate (4) and by an enzyme in pregnancy plasma which is known to destroy ADH (5); 2) during suckling the antidiuretic activity of the extracts increases; and 3) the slopes of the regression lines for standard ADH (Pitressin) and for the antidiuretic substance in the extracts were parallel (Fig. 1).

There is less ADH in the blood of non-pregnant females than in pregnant women towards the end of term. This confirms a view previously reported by one of us (5, 6). In the 2 cases of labor investigated, and in the women before suckling, the levels of ADH in the blood were of the order found during pregnancy, whereas during suckling there was a two-fold increase.

Summary. Using a modification of Bisset and Walker's method, ADH has been extracted from venous blood collected from women during non-pregnant states, pregnancy, labor, and before and during suckling.

The concentration of ADH in non-pregnancy blood was considerably

TABLE 1. ANTIDIURETIC ACTIVITY OF ACID-ALCOHOL EXTRACTS OF BLOOD IN NON-PREGNANT STATES, PREGNANCY, LABOR, AND BEFORE AND DURING SUCKLING. INACTIVATION WITH SODIUM THIOGLYCOLLATE WAS COMPLETE IN ALL CASES. BLOOD WAS COLLECTED TEN MINUTES BEFORE SUCKLING AND AGAIN FIVE MINUTES AFTER SUCKLING COMMENCED (*i.e.*, DURING SUCKLING)

Patients	ADH in blood (μU/ml.)	
Non-pregnant		
1	9	
2	<6	
3	<8	
4	16	
5	<6	
6	<28	
7	<10	
8	<10	
9	22	
10	<10	
	Avge. *<12.5*	
Pregnant		
1	43	
2	29	
3	21	
4	34	
5	64	
	Avge. *38*	
Labor		
	48	
	37	
	Avge. *42*	
Suckling	Before	During
1	73	110
2	33	60
3	25	85
4	23	33
5	23	44
	Avge. *35*	*66*

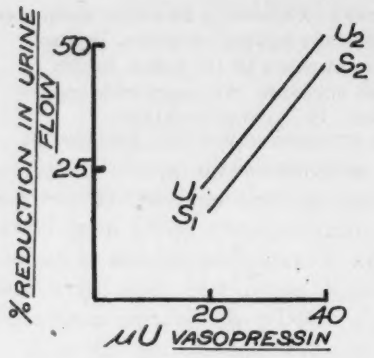

FIG. 1. Showing parallelism between the slopes of the regression lines for standard ADH (Pitressin) and for the antidiuretic substance in the blood extracts. *Abscissa:* log dose in micro-units of vasopressin (Pitressin). *Ordinate:* antidiuretic effect. S_1 and S_2 are the responses for the low and high doses of standard. U_1 and U_2 are the responses for the low and high doses of unknown. Each point represents the mean of 4 results. The estimated potency ratio was 1.45; the actual potency ratio was 1.50.

less than that found during pregnancy, labor, or before suckling; during suckling the concentration of ADH was almost doubled.

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LACK OF ANDROGENICITY OF 17-HYDROXY-PROGESTERONE OR ITS 17-CAPROATE ESTER

To the Editor:

The recent review by Reifenstein (1) on the subject of 17-hydroxyprogesterone and its presumed role in the pathogenesis of the adrenogenital syndrome emphasizes the importance of ascertaining the androgenic properties of this steroid. The various animal assays to which this compound has been subjected are detailed in his review, and do not give the impression that it is a potent androgen. However, the matter of species differences is at times an important one, and we began some time ago to explore, among other things, its androgenic potency in the human. For this aspect of the problem, two types of patients were selected in whom androgen effect might be particularly easy to observe or in whom the sensitivity to androgen might be particularly high.

Two boys, each 14 years of age, with slight statural and marked sexual retardation comprised one group. *Patient W.S.* had a high voice and no axillary, pubic or facial hair. The penis was small; the testes (descended) were comparable in size to those of a 10-year-old boy. The 24-hour urinary ketosteroid excretion averaged 11.7 mg. and the corticoid excretion, 6.7 mg. He was given a single oral dose of 100 mg. of 17-hydroxyprogesterone daily for a total of eighty-four days, during which time urine was collected at intervals for various studies. Serial photographs of the pubic region, axillae and face were taken with a Coreco clinical camera, which assures identical area, magnification and exposure. This was hardly necessary, since no pubic or axillary hair developed, and there was no change in the voice. Subsequently, he was given testosterone enanthate[1] and underwent prompt masculinization. *Patient R.K.*, the second boy, manifested somewhat more marked statural retardation, but was otherwise similar to Patient W.S. in having a high voice and no pubic, axillary or facial hair. He received 200 mg. of 17-hydroxyprogesterone caproate[2] daily by mouth in divided dosage for a total of sixty days. There was no perceptible androgenic effect as recorded by serial photographs. For an additional twenty-day period he was given 200 mg. a day of free 17-hydroxyprogesterone orally—also without effect. He then received 9 injections (132 mg. each) of a microcrystalline suspension of 17-hydroxyprogesterone at three-day intervals. Ten days after the last injection there had been no sign of change in secondary sexual characteristics. Following this, he was given 10 injections (1000 I.U. each) of chorionic gonadotropin at

[1] Delatestryl (Squibb).
[2] Delalutin (Squibb).

three-day intervals, whereupon his voice changed promptly and pubic and axillary hair developed

Two cases of hypopituitarism were selected for the evaluation of androgenic changes in the female. *Patient U.R.* was a 25-year-old female in whom Sheehan's syndrome developed following her first pregnancy. There was mild hypothyroidism (which was corrected with 10 micrograms of trioiodothyronine daily) and a tendency to hypoglycemia. The 24-hour urinary excretion of 17-ketosteroids was 3.6 mg., and of corticoids, between 0.5 and 1.3 mg. The patient's pubic or axillary hair had not grown in again following its disappearance in the postpartum period. On pelvic examination there was a minute clitoris, and the vaginal smear showed very slight evidence of estrogen production. In the first study period she received 200 mg. of 17-hydroxyprogesterone orally per day in two doses, for a total of forty days. Serial Coreco photographs failed to reveal an increase in clitoral size or any growth of pubic or axillary hair. After a control period of two weeks, she received 200 mg. by mouth daily for an additional twenty-one days. No androgenic changes occurred during this second course of therapy. The second patient, *V.S.*, was a 31-year-old physician who presented a classic picture of hypopituitary dwarfism. She was less than 5 feet tall, was totally devoid of secondary sexual characteristics, and had typical young-old facies. The 24-hour excretion of urinary 17-ketosteroids was 5.5 mg., and of corticoids, 2.9 mg. There was some response to a single dose of corticotropin. There was no pubic or axillary hair, and the clitoris was minute. A vaginal smear showed a picture of total estrogen deficiency. A course of 17-hydroxyprogesterone in a daily dose of 200 mg. orally for a total of twenty days failed to produce any changes, and a second course of the caproate ester orally in the same dosage for a period of forty days was likewise ineffective.

A further aspect of our studies may also deserve mention. The potency of 17-hydroxyprogesterone or its caproate ester with regard to inhibition of pituitary production of corticotropin was studied in 3 patients with adrenal hyperplasia. *Patient G.S.* was a 31-year-old woman who had had a clitoridectomy at the age of 2 and one adrenal removed at the age of 25. At the age of 30 her control urinary 17-ketosteroid excretion ranged between 65 and 100 mg. per day, and corticoid excretion was between 5.6 and 9.3 mg. per day. A daily dose of 10 mg. of prednisolone reduced the 17-ketosteroid excretion to 8–11 mg. per day. After an adequate control period, she was given 300 mg. of free 17-hydroxyprogesterone daily by mouth for six days, followed by 200 mg. daily for twenty days. No depression of urinary 17-ketosteroid excretion was observed. She was then given 250 mg. of the caproate in oil twice weekly for a total of 5 injections; again no response was observed. Resumption of oral cortisone therapy decreased

the urinary 17-ketosteroids to normal levels. Subsequently, *2 young women* with the chief complaint of hirsutism were also tested. Their urinary 17-ketosteroid excretion ranged from 18 mg. to 19 mg. per day, and was decreased to the normal range of 6 to 11 mg. per day by means of small oral doses of cortisone or hydrocortisone. Such treatment also had a pronounced inhibiting effect on the facial hair growth. After stopping steroid therapy and allowing the urinary 17-ketosteroid excretion to return to initial values, these patients were given 250 mg. of 17-hydroxyprogesterone caproate intramuscularly once a week for three and six weeks, respectively. This treatment failed to have any effect on the urinary 17-ketosteroids. The free compound was not tested, as both patients insisted on resumption of the previous treatment.

Jailer *et al.* (2) showed that oral administration of 17-hydroxyprogesterone is followed by urinary excretion of its metabolites; therefore it is likely that intestinal absorption occurs, to some extent at least. If this be correct, our studies indicate an absence of significant androgenic effect in the human, even after substantial and prolonged doses of 17-hydroxyprogesterone or its caproate ester orally. The results in the one instance in which the free substance was given by intramuscular injection also confirm these findings. In our studies, neither the free compound nor its caproate ester seemed to exert any influence on pituitary overproduction of corticotropin in benign adrenal hyperplasia. It would, therefore, appear that neither 17-hydroxyprogesterone nor one of its direct metabolites accounts for the androgenic effects seen in adrenal hyperplasia.

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Acknowledgment

We should like to express our appreciation to Dr. E. C. Reifenstein, Jr. of E. R. Squibb and Sons for ample supplies of Delatestryl, Delalutin, and free 17-hydroxyprogesterone used in this study.

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DIURNAL 17-KETOSTEROID EXCRETION AND ITS APPLICATION TO STRESS AND THE AGING PROCESS

TO THE EDITOR:

In a recently published book, Pincus (1) offers us a fact-filled survey of the major steroid hormone derivatives excreted in the urine on a time-track schedule—diurnal variation.

Over the past five years this writer has studied the pattern of day and night versus total 24-hour excretion of 17-ketosteroids (17-KS) in several hundred subjects of both sexes, from the first to the seventh decade of life. In a significant number of cases (37 per cent), an irregular rhythm of 17-KS output for the night specimen led to the conclusion that the concept of an average tendency toward a "diurnal rise" is invalid for some individuals. The following 2 cases are offered as illustrations of the difficulty of applying such data for the purpose of clinical interpretation or the development of physiologic postulates.

Case 1. This normal woman, aged 43, was married and had 2 children. Following the first series of collections of urine, she volunteered the information that she always had a difficult time getting up in the morning since childhood. For this reason, the diurnal 17-KS determinations were repeated (and in all subsequent cases that yielded similar findings). Her retiring-time prior to, and during the studies was around midnight. Instructions on the procedure for collecting the urine specimens were as follows: "The first morning sample is to be cast away; then every sample (urination) during the day prior to 8 P.M. is to be placed in the bottle marked 'day'; samples after 8 P.M., and during any time of the night, including the following morning sample, are to be placed in the bottle marked 'night'." This corresponds to the typical method of collecting a 24-hour specimen, except for the arbitrary division into two separate containers at 8 P.M. The data for 17-KS determinations on three alternate successive split 24-hour specimens of urine are tabulated in Table 1.

The study was repeated the following month when the subject could indulge her desire to sleep longer and later into the morning, compared with the sleeping period during the first series of collections. The only change in the collection schedule was that the specimen collected at the time she spontaneously awoke (11:10, 11:30 A.M., or 12:00 noon) corresponded to the "early morning" voiding in previous studies. The results of the second group of split 24-hour 17-KS determinations are tabulated in Table 2.

TABLE 1. CASE 1. 17-KS IN FIRST SERIES OF SPLIT 24-HOUR URINE COLLECTIONS

17-KS in total 24-hour specimen (mg.)	17-KS in day specimen (mg.)	17-KS in night specimen (mg.)
8.4	6.9	1.5
8.8	7.2	1.6
8.0	7.0	1.0

TABLE 2. CASE 1. 17-KS IN SECOND SERIES OF SPLIT 24-HOUR URINE COLLECTIONS

17-KS in total 24-hour specimen (mg.)	17-KS in day specimen (mg.)	17-KS in night specimen (mg.)
9.6	3.4	6.2
10.0	3.5	6.5
7.6	3.6	4.0

Case 2. This subject was a normal young man, aged 19. There was no history of difficulty in getting up in the morning. The urine collection schedule was identical with that for the first study in Case 1. He rose early to attend school, and also retired at a late hour. The urinary 17-KS determinations on the split 24-hour specimens in this subject are listed in Table 3.

The urinary 17-KS estimations were carried out on residues from acid-hydrolyzed aliquots under benzene, treated with the Zimmermann reagents.

COMMENT

In Case 1 the disparity in nightly 17-KS excretion between the two studies (Tables 1 and 2) is striking. More interesting is the contrast between the nightly 17-KS excretion for Case 1 (first series, Table 1) and that for Case 2 (Table 3) collected according to the same time-schedule. Since there was no nocturia in either of the 2 subjects, it may be assumed that the morning urination represented the total urine output after retiring.

The whole question of the "diurnal rise" in steroids has been brought to the fore by the recently published book by E. T. Engle and G. Pincus entitled "Hormones and the Aging Process" (1). In the first chapter, under the heading: "Aging and the Urinary Steroid Excretion," Pincus discusses the diurnal rise of 17-KS in relation to stress, and the aging process in general. Unfortunately, although such terms as "A.M." and "P.M." are used in the text, the terms "Waking," "Sleeping" and "Day" are used as

TABLE 3. CASE 2. 17-KS IN THREE SUCCESSIVELY ALTERNATE SPLIT 24-HOUR URINE COLLECTIONS

17-KS in total 24-hour specimen (mg.)	17-KS in day specimen (mg.)	17-KS in night specimen (mg.)
18.4	8.0	10.4
19.3	6.3	13.0
16.7	5.2	11.5

column headings in the summary of data in the tables, thus making it very difficult to coordinate the values relevant to A.M. and P.M. terminology. Pincus favors the localization of the excretory rise to the daytime. However, because of the mixed terminology, it is not clear what is meant by "day," "sun-rise" or "subject-rise." It is obvious from the sequence of 17-KS outputs described in our present report that the 2 subjects did not maintain the same constancy of internal environment. The 17-KS excretion pattern in Case 1 (Table 2) resembled that in Case 2 (Table 3) only after a shift in the "time-track" for awakening, from 6–7 A.M. to 11 A.M.–12 noon. Pincus (1) attributes the so-called "diurnal rise" to an attempt of the pituitary-adrenal system to rise to the occasion of adjusting to the "waking life." Obviously, in the case of the female subject described here, the hormonal internal environment was not adequately in tune with the external environment when she was compelled to awaken at an early morning hour.

It is not practical, outside of institutional or hospital residence, to carry out 2-hour or 3-hour collections of urine. In view of the importance of the stereo-pattern of the "diurnal rise" in 17-KS output, a practical uniform schedule of collections for non-hospitalized ambulatory subjects should be agreed upon. Thus the observed phenomena can be subjected to a reasonable degree of mathematical formulation of the vectors, time, and degrees of increase in night versus day excretion of 17-KS, in order to establish a constant of the type that may have clinical and physiologic implications.

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METABOLIC EFFECTS OF INTRAVENOUS TRIIODOTHYROACETIC ACID IN PRIMARY MYXEDEMA

TO THE EDITOR:

In several studies it has been shown that there is dissociation of the effects of triiodothyroacetic acid on the basal metabolic rate (BMR) and

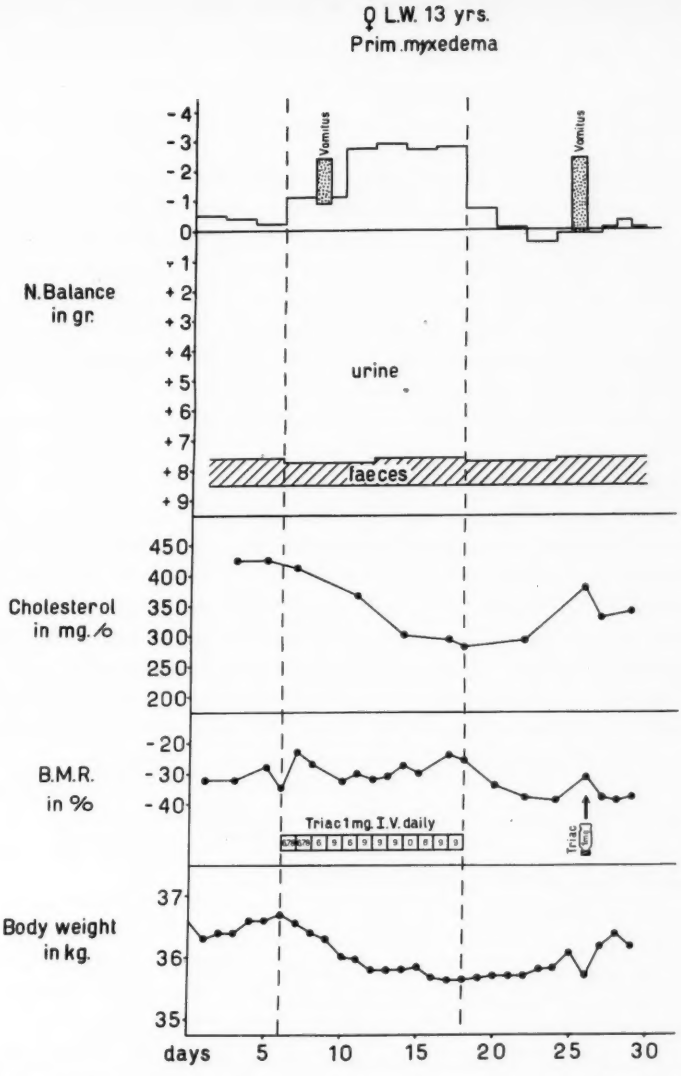

FIG. 1. Metabolic effects of intravenous triiodothyroacetic acid in a 13-year-old girl with primary myxedema. The BMR was always measured at 9 A.M. The administration of 1 mg. of triac intravenously was varied in the following ways with regard to dosage, and relative to the time of the BMR.:

1. In doses of 1/3 mg., each at 6, 7 and 8 A.M. the same day.
2. In a single dose of 1 mg. at:
   a) 9 A.M. the preceding day (interval 24 hours).
   b) At 0 A.M. the same day (interval 9 hours).
   c) At 6 A.M. the same day (interval 3 hours).
   d) At 8 A.M. the same day (interval 1 hour).
3. By infusion lasting from 3 A.M. to 9 A.M. the same day.

on the level of blood cholesterol (1–3). We have found a similar dissociation of effects on the BMR (oxygen consumption) and on other metabolic parameters (blood cholesterol level, and excretions of nitrogen, creatine and phosphorus).

Triiodothyroacetic acid (triac) was administered in a dosage of 1.0 mg. intravenously daily for a period of twelve days to a 13-year-old girl with primary myxedema. There was no clear-cut effect on the BMR, even when the interval between the administration of triac and estimation of the BMR was varied, or when triac was given in divided dosage (Fig. 1). Eight days later, 1 mg. of triac was given by intravenous infusion over a period of six hours. This also did not have any effect on the BMR, as measured during and after the infusion.

During treatment with triac, the blood cholesterol level fell from 430 mg. to 280 mg. per 100 ml. (Fig. 1). Thus we are able to confirm the findings of Lerman and Pitt-Rivers (3) in this regard.

As seen in Figure 1, triac had a pronounced effect on nitrogen balance. Nitrogen balance was strongly negative during the period of administration of triac, but there was a reversion to equilibrium immediately after the cessation of treatment. During the same period the excretion of phosphate and creatine increased and electrocardiographic findings reverted toward normal. Further details will be published later.

This study suggests that the general metabolic effects of triac are comparable to those of thyroxine, with the exception that there is no change in the basal metabolic rate (oxygen consumption).

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We are indebted to Dr. R. Pitt-Rivers, F.R.S., of the National Institute of Medical Research and to Glaxo Laboratories Ltd., Greenford, Middlesex, for the supply of triac.

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USE OF AN ORGANIC BASE IN THE ZIMMERMANN REACTION*

To the Editor:

In the course of a systematic examination of several organic bases as a substitute for potassium hydroxide in the Zimmermann reaction, N-benzyl trimethyl ammonium methoxide has presented certain noteworthy advantages. It is stable in solution for several months, reagent blanks develop less color than with potassium hydroxide, there is a lower incidence of spurious atypical color development, and most standard supplies of absolute ethanol may be employed without redistillation.

N-Benzyl trimethyl ammonium methoxide is supplied as a 40 per cent solution in methanol (Sumner Chemical Company, New York, N. Y.). We have employed it at this concentration, as supplied, and in various dilutions with ethanol and methanol. The most satisfactory results and the maximum sensitivity have been obtained with the 40 per cent methanolic solution. Otherwise, ethanol was used for purposes of dilution and for the preparation of a 2 per cent solution of *m*-dinitrobenzene. Appropriate residues containing 17-ketosteroids were dissolved in 0.2 ml. of absolute ethanol, to which 0.2 ml. of a solution of *m*-dinitrobenzene was added. After immersing all tubes in ice water, 0.3 ml. of cold 40 per cent N-benzyl trimethyl ammonium methoxide was added and the color developed at 4° to 8° C. for five hours. All tube contents were then diluted with 1.0 ml. of 50 per cent ethanol and the optical density determined at various wave lengths in the Coleman Junior spectrophotometer. Longer periods of color development may be employed at the lower temperature to suit the convenience of the laboratory. The procedure outlined is modified after Wilson (1) and is designed as a "micro" technique, but the final dilution may be increased to meet the conditions of the concentration of steroids to be measured and the spectrophotometer. However, it is important that 50 per cent ethanol serve as the diluent, since higher concentrations frequently lead to the appearance of a flocculent precipitate. The stability of the color after dilution is similar to that with ethanolic potassium hydroxide. Using the outlined technique, we have found the sensitivity to be equal to that obtained with ethanolic potassium hydroxide. The absorption maximum of the color obtained has been in the range 510–515 mμ, regardless of whether ethanolic potassium hydroxide or N-benzyl trimethyl ammonium methoxide was used, as determined with the Beckman DU spectrophotometer.

* This work has been supported by a grant (A-619 (C2)) from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service, and by a Grant-in-Aid from the American Cancer Society.

Reagent blanks have yielded consistently low readings with the organic base, whether or not the ethanol used had been redistilled. Certain batches of absolute ethanol known to produce high blanks with potassium hydroxide in the method described, were selected for purposes of comparison. When the optical densities of such blanks were 0.234–0.286 with potassium hydroxide, they were only 0.020–0.028 with the organic base—the instrument being set at zero for ethanol alone. We have no longer been obliged to redistill shipments of absolute ethanol which proved unsuitable with potassium hydroxide. However, deliberate contamination with small quantities of acetone produced intense brown color with either base.

It is advisable to keep containers of new base tightly stoppered, but we have obtained the described results with the solution of the organic base for several months after breaking the metal seal on the original package. A small amount of sediment may appear after a few weeks, but this does not interfere with the reaction.

The application of N-benzyl trimethyl ammonium methoxide to the Zimmermann reaction on paper chromatograms has also proved advantageous. One part of the 40 per cent solution is mixed with one part of absolute ethanol and two parts of 2 per cent *m*-dinitrobenzene in ethanol. It appears that with heating (about 90° C.) the color development is enhanced and the background is minimized. Higher concentrations of the organic base may produce an intense red color throughout the paper.

Further details of these studies will be published later.

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Acknowledgment

The authors are indebted to Doctor Peter DeBenneville for helpful advice.

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The Endocrine Society

THE 1957 ANNUAL MEETING

The Thirty-ninth Annual Meeting of The Endocrine Society will be held in the Hotel New Yorker, New York City, Thursday, Friday and Saturday, *May 30 and 31, and June 1, 1957.*

A joint session will be held with The American Goiter Association on Thursday morning, May 30, and a joint session with The American Diabetes Association on Saturday morning, June 1. Both of these joint sessions will be held in the meeting room of The Endocrine Society in the Hotel New Yorker.

The Committee on Local Arrangements is Dr. Rulon W. Rawson as Chairman with Drs. Earl T. Engle, Joseph W. Jailer, Warren O. Nelson, and Martin Sonenberg as members of the Committee.

All Scientific Sessions will be held in the Hotel New Yorker. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, May 31, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the New Yorker, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

Those wishing to present papers, which will be strictly limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Eleanor Venning, Royal Victoria Hospital, Montreal, Quebec, Canada, *not later than February 1, 1957. It is imperative that the abstracts be informative and complete with results and conclusions—not a statement that teese will be presented at the meeting*—in order that they may be of reference value and suitable for printing in the program and journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. Reference, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.

2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each nonmember author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.
   Line 3. Institution of origin and city in which institution is located.
3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter perfect, since there will be no opportunity for proof reading by authors.

THE 1957 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955 and Dr. Frederick L. Hisaw in 1956.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs.

Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any mem-

ber of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

The American Goiter Association

THE 1957 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Statler Hotel, New York City, on *May 28, 29 and 30, 1957.*

THE AMERICAN GERIATRICS SOCIETY

The next Annual Meeting of the American Geriatrics Society will be held in the Waldorf-Astoria, New York, N. Y., *May 30 and 31, 1957.* Inquiries may be addressed to Dr. Richard J. Kraemer, Secretary-Treasurer, 2907 Post Road, Greenwood, R. I.